THE

AMERICAN NATURALIST

Established 1867

A BI-MONTHLY JOURNAL

Devoted to the Advancement and Correlation
of the Biological Sciences

Edited in the interest of The American Society of Naturalists

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Published for
THE AMERICAN SOCIETY OF NATURALISTS
by
THE SCIENCE PRESS
Lancaster, Pennsylvania
1956

THE SCIENCE PRESS
LANCASTER, PA.

THE AMERICAN NATURALIST

THE
AMERICAN NATURALIST

Vol. XC January–February, 1956 No. 850

MODES OF SELECTION*

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INTRODUCTION

In order to get to the topics which I wish to discuss, it will be necessary to begin with a rather dogmatic statement of my general position with respect to evolution.

Adaptation rather than mere change seems to me to be the central problem. The only mechanism for evolutionary adaptation that has held up under investigation is natural selection.

Selection can not, of course, operate without raw material in the form of undirected genetic variability. This may include both inconspicuous and conspicuous gene mutations, chromosome aberrations of all sorts, genetic material from genes to genome introduced by hybridization, genotypes as wholes produced by recombination, and differences among populations from local ones within species to competing species and higher categories. All of these are raw materials which require natural selection for assemblage into patterns that constitute significant evolutionary change.

The topic which I discuss is a comparison of the modes of selection at various levels of organization.

SELECTION AMONG GENES AND CELLS

At the lowest level is selection among genes in mere capacity to persist and in mechanism of exact duplication. It seems probable, as suggested by Darwin (cf. Hardin 1950) and developed by Haldane (1933) and Oparin (1938), that life originated in an organic soup that could only have accumulated in the prior absence of life and that was one that provided all necessary metabolites. It is hardly probable that the chemical basis for gene persistence and duplication appeared full fledged. We may conclude with Blum (1951) that evolution took the form at first of decreasing mutability. Along with this may have occurred the evolution of stable genic association in cells, and mechanisms of exact duplication of the entire system by mitosis.

*Address of the President, Society for the Study of Evolution, delivered at the annual meeting, Austin, Texas, April 10, 1955.

With the attainment of a sufficient degree of stability in these respects, but increasing depletion of natural resources, selection for capacity to take necessary metabolites from other organisms, or to synthesize them from precursors would take precedence over selection for further stability (Horowitz, 1945). Decreasing mutability would, however, continue to be important whenever evolutionary increase in the length of the life cycle occurs.

SELECTION UNDER EXCLUSIVE UNIPARENTAL REPRODUCTION

Exclusive uniparental reproduction leads to the formation of clones of constant genotype, except for rare mutations. Selection between clones is an extremely effective process since the selective value of the genotype as a whole is the basis. Even very slight differences in rate of reproduction, in a limited environment, leads rapidly to extinction of all but the most rapidly multiplying clone (Haldane 1932). Thereafter, however, the material for evolution is restricted to the mutations that arise from time to time. Of two mutations that are present at the same time in a competing population, only one persists. Recombination requires mutation in tandem in the same line.

MASS SELECTION

Biparental reproduction gives the great advantage that the evolution of the entire population, rather than a single lineage, is promoted by selection. Mutations that arise in different individuals may later be combined. A store of variability is maintained in various ways. Evolution may occur simultaneously in all characters.

The ways in which the frequencies of favorable mutations increase in a great variety of circumstances were presented by Haldane in a series of early papers summarized in 1932.

THE PLEIOTROPIC THRESHOLD

An important question is whether there is a threshold in the selective advantage of a gene over its alleles which must be exceeded for effective operation of natural selection. As far as over-all selective advantage is concerned, it is clear enough from Haldane's analysis that there is no such threshold, although selective difference of, for example, 10^{-6}, would require a great many generations to produce an appreciable change in gene frequency.

Selection of a particular character is, however, a very different matter. This is because of the prevalence of pleiotropy, using the term in the broad sense to include not only primary pleiotropy (effects of the gene itself on more than one process) but also secondary pleiotropy (effects on different characters that branch out from a single primary effect). The epigenetic nature of development implies secondary pleiotropy as an almost universal principle. Inspection of such lists of mutant effects as given by Bridges and Brehme (1944) for *Drosophila melanogaster* indicates that this is in fact the case, even at the level of gross effects on viability, fecundity,

color and morphology. It is probable that investigation focused on this question would reveal pleiotropic differences for all distinguishable alleles.

If we analyze the total selective value (W) of a gene into terms (W_1, W_2, W_3, etc.) that relate to characters that are known to evolve largely independently, total selective value may be considered as the product of the components, or in terms of selective advantage ($S = W - 1$), the total is approximately the sum of the contributions, assuming that all S's are small. If $S_1 = -.0001$ and $S_2 = +.01$, it is obvious that the gene will tend toward fixation because of the favorable effect on the second character, in spite of its unfavorable effect on the first. If $S_1 = -.0001$ and $S_2 = -.01$ it will tend toward extinction, but not to an important extent because of its unfavorable effect on the first character. In the class of genes that have the selective disadvantage 10^{-4} for a particular character, this effect decides the issue only for those for which the coefficient for residual effects is less than 10^{-4}.

These considerations probably have an important bearing on the complete loss of useless characters or their persistence as vestiges (Wright 1929). No doubt selection against such characters as encumbrances is the most important factor in their degeneration up to a certain point. Beyond this, selection based on pleiotropic effects may be expected to take over. The genes on which the useless character depends may be expected to be involved in other characters. When the direct value to the organism of further degeneration becomes small enough, persistence or loss depends largely on the selection coefficients relative to these. If the organism is conservative in these respects, the favorable selection coefficients may maintain the useless character as a vestige for a very long time, in spite of a slight negative component for the effect on the useless character by itself. If, on the other hand, there is considerable reorganization of these other characters, the selection coefficients favoring the replacing alleles will tend to bring about relatively rapid complete loss of the useless character as a byproduct.

The pleiotropic threshold also has an important bearing on theories of the evolution of dominance (Wright 1929, 1934), a matter on which my position has often been misrepresented. (e.g. in Fisher 1949 and earlier)

MULTIPLE PEAKS IN SELECTIVE VALUE

The most serious difficulty with mass selection as the primary guiding principle in evolution is that it does not apply to the genotype as a whole but only to the net effects of the individual genes taking account of all of the genotypes in which they enter. This is because genotypes, formed in one generation, are broken up in the next, by the reduction division, and the random union of gametes.

This limitation would not be serious if each gene replacement always contributed to selective value in the same sense in all combinations, since in this case there would always be just one goal (in terms of the genes currently present) toward which the species would move under mass selec-

tion from any initial composition. The same result would occur even if there were no contribution in some combinations with genes at other loci or even if there were reversal of effect in isolated cases.

There may, however, be two or more peaks, even at a single locus, such that mass selection may lead to a lower peak from certain initial states and hold the population there the more firmly, the more severe the selection. The simplest case is, of course, that of two alleles with a heterozygote inferior to both homozygotes. This is probably not very common. The case of semi-sterility due to reciprocal translocation can, however, be treated formally as of this sort in plants in which the aneuploid gametophytes are eliminated. The case in animals is more complicated but somewhat similar and will be discussed later.

A situation that is probably of very considerable evolutionary importance may occur in the common case in which superiority of the heterozygote A_1A_2 of two alleles maintains a population in a heterallelic state. A mutation A_3 may occur which combines the favorable effects of A_1 and A_2, and if fixed would produce a population superior to the previous heterallelic one (Haldane 1937). If the heterozygotes A_3A_1, A_3A_2 (assumed equal in selective value) have more than half the selective advantage of the original heterozygote A_1A_2 over the two homozygotes A_1A_1 and A_2A_2 (also assumed equal), all three alleles are retained (or A_3 is fixed), in a population with a single peak. If however A_3A_1 and A_3A_2 have less than half the advantage of A_1A_2 over A_1A_1 and A_2A_2 there are two peaks. Mass selection may keep the population essentially heterallelic for A_1 and A_2 only, (in spite of mutations to A_3), even though a population homallelic in A_3 would be superior (based on formula in Wright 1949a).

Similar situations can occur in combinations of two pairs of alleles. It could happen, for example, in the selection of guinea pigs for a rosette on the forehead. Assuming a certain common genetic background (*mm*), genotype *rr stst* has no forehead rosette, *R-stst* has a rather well developed one, *rr St-* has a very well developed one, while the combination *R-St* has only a slight irregularity in this region (Wright 1950).

There are other cases of this sort in the guinea pig, but it must be admitted that a list of such special cases does not indicate that factor interaction would often interfere seriously with progress under mass selection.

If, however, we focus on selective value of the genotype as a whole, as we must in dealing with natural selection, in contrast with artificial selection for special characters, it may be seen that a multiplicity of peak genotypes may be expected to be the rule.

In any species that has been exposed for a long time to similar conditions, the optimum grade of most quantitatively varying characters is to be expected near the mean. Assuming determination of the grade by the cumulative effects of multiple factors as in the conventional theory, the same gene replacement that is favorable in genetic backgrounds sufficiently below the optimum is unfavorable in ones above. Grades, near the optimum, are given by a number of different homozygous genotypes, differing by two

or more steps and thus at independent peaks in the surface of selective values. Mass selection tends to fix one of these, according to the initial gene frequencies, or at most, to maintain one locus in a heterallelic state (Wright 1935).

It may seem of little evolutionary significance which peak is attained since all have about the same selective value by hypothesis, but this is only according to the effects on this particular quantitative character. Taking pleiotropic effects into account, there may still be multiple peaks, but with widely different selective values.

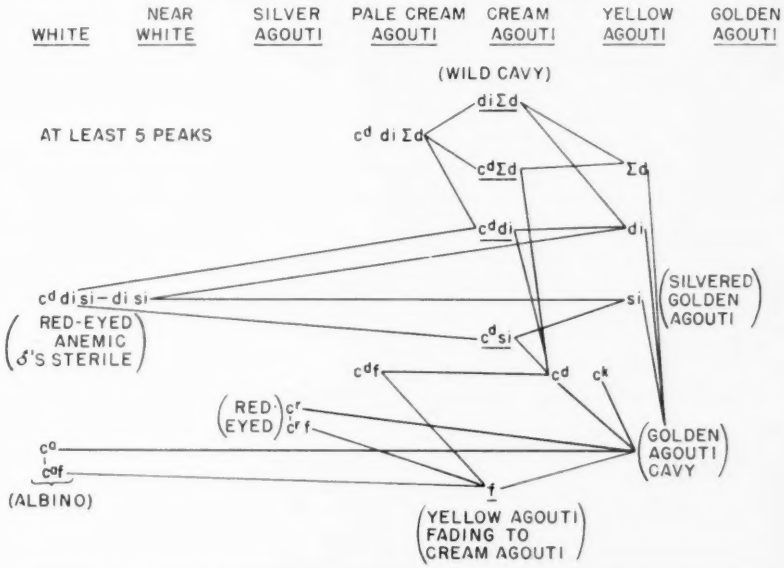
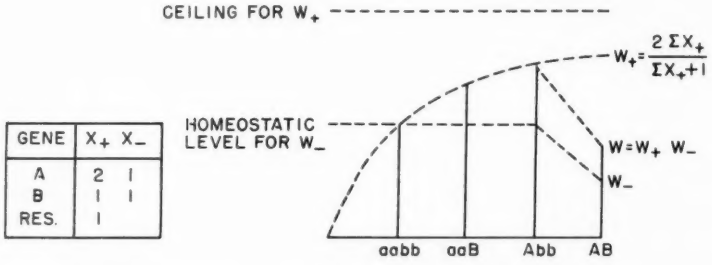


FIGURE 1. Certain genotypes of the guinea pig and wild Peruvian Cavy *(Cavia cutleri)* according to difference from the golden agouti variety. The protectively colored cream agouti color of the wild cavy is definitely not due to an albino allele (c^dc^d or c^kc^k), which gives cream agouti in association with minor dilution factors (such as *si* and *di*). It differs from the golden agouti by multiple minor factors (Σd) possibly including the factor *di* of the guinea pig. There are at least 5 adaptive peaks due to this intermediate optimum.

Figure 1 is a simplified illustration of such a case. The color of the Peruvian Cavy, *Cavia cutleri*, the probable wild ancestor of the guinea pig, is like the guinea pig color known as cream agouti (Castle in Castle and Wright, 1915). It is well adapted for protective concealment. It is intermediate between silver agouti (or the even more extreme white) and the intense golden agouti color. Cream agouti guinea pigs, resembling more or less closely the color of the wild cavy, can be produced in many ways, but the results of crosses indicate that none of these is as close genetically as is the golden agouti. There are probably at least five homozygous cream agouti genotypes each at two or more removes from each of the others

(Wright, 1949b). All are thus at different adaptive peaks in the surface of mean selective values with respect to protective coloration. There are, however, differences in the intensity of the sepia in the hair, even though the subterminal cream band is the same, and differences in the amount of fading of later pelages, that make it certain that not all peaks would have the same adaptive value.

Even if the optimum grade of a character is beyond the current range, pleiotropy can easily give rise to multiple peak genotypes. A number of model cases are illustrated in figures 2 to 8. The simplest is figure 2. Dominant genes A and B are supposed to contribute cumulatively but unequally to the grade of a favorable character, and also cumulatively, and in this case equally, to an unfavorable one. Residual factors (Res.) contribute only to the former and are easily brought to near-fixation by selection. It is assumed that there is damping of the favorable contributions as the totals

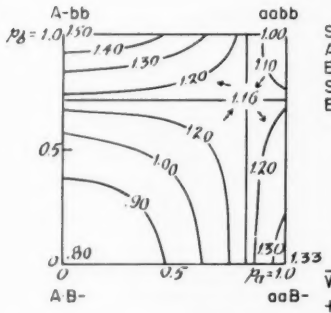


GENE	X_+	X_-
A	2	1
B	1	1
RES.	1	

FIGURE 2. Genes *A*, *B* (and residual heredity Res.) contribute favorably (X_+) to one character, but *A* and *B* tend to contribute unfavorably (X_-) to another. The component of selective value due to the former (W_+) is subject to damping as a ceiling of possible favorable effect is approached. The component (W_-), due to the latter, is under homeostatic control with respect to the effects of *A* and *B* separately, but this breaks down in the combination *AB*. Populations homallelic in *AAbb* and *aaBB* are at distinct peaks in net selective value (W).

increase, at least in selective value, in accordance with the almost universal principle of diminishing returns from increasing application of any one kind of factor. Another sort of factor becomes the limiting one. This is analogous to the theory of dominance and of the extreme lability of intermediate heterozygotes (Wright 1929) but is here applied to combinations of loci (Wright 1934). Rasmussen (1933) has discussed such damping in connection with crop yields.

The tendency toward unfavorable effects by *A*- or *B*- each by itself, is assumed to be prevented by homeostasis built up in the past against this sort of effect, but it is assumed that the combination *A*-*B*- deviates more than can be taken care of in this way. The result with the selective values chosen is that *aaB*- and *A*-*bb* are peak genotypes at different levels, while *A*-*B*- is even less well adapted than *aabb*. Figure 3 shows the surface of mean selective values, $\overline{W}$, by means of contours.



MEAN SELECTIVE VALUE IN SIMPLE CASE OF PLEIOTROPY ASSUMING DAMPING OF FAVORABLE EFFECT AND HOMEOSTASIS FOR SINGLE DOSE OF UNFAVORABLE EFFECT.

GENE	X_+	X_-
A	2	1
B	1	1
RES.	1	

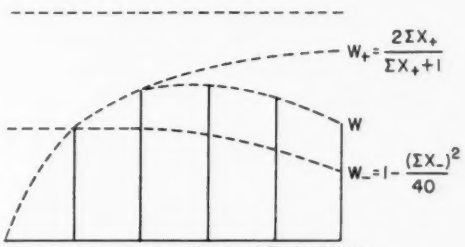
$$\bar{W} = 1.00\,P_a^2P_b^2 + 1.33\,P_a^2(1-P_a^2) + 1.50\,(1-P_a^2)\,P_b^2 + .80\,(1-P_a^2)(1-P_b^2)$$

FIGURE 3. The contours on the surface of net selective value (W) in the case shown in figure 1.

It is evident that mass selection might lead to either one of these peaks, depending on the initial composition of the population, and, if the lower one, it would prevent attainment of the higher one. Artificial selection for the favorable character would, on the other hand, tend toward fixation of genotype *AABB* with disastrous effects with respect to the unselected character. It is notorious that strong artificial selection usually has this sort of consequence (cf. Mather and Harrison 1949, Lerner and Dempster, 1951, Robertson and Reeve 1952a and many others).

Figure 4 represents a case in which exclusively favorable residual heredity is supplemented by four dominant factors with equal favorable effects, and also with equal unfavorable effects on a second character. The law of diminishing returns is again assumed to apply to the favorable character and homeostasis to the unfavorable one. The chosen values result in a situation similar to that with an intermediate optimum. With four pertinent

GENE	X_+	X_-
A	1	1
B	1	1
C	1	1
D	1	1
RES.	1	



2 PITS
6 PEAKS

FIGURE 4. Similar to figure 1 except that four dominant factors contribute favorably to one character, subject to damping, and unfavorably to a second, subject to decreasing homeostatic control, in both cases as the cumulative primary effects increase.

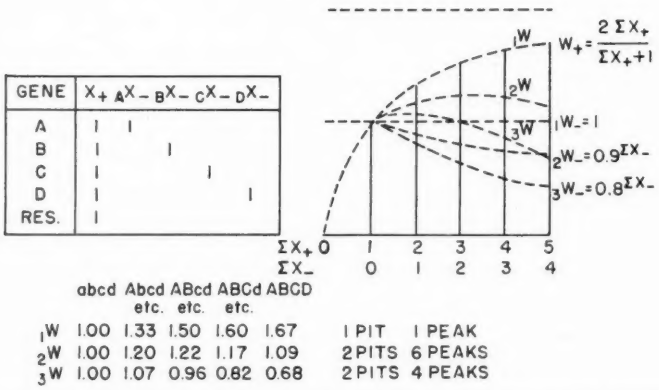

GENE
X+ AX− BX− CX− DX−
A
B
C
D
RES.
1W
2W
3W
W+ = 2ΣX+ / ΣX+ +1
W− = 1
2W− = 0.9^ΣX−
3W− = 0.8^ΣX−
ΣX+ 0 1 2 3 4 5
ΣX− 0 1 2 3 4
abcd Abcd ABcd ABCd ABCD
etc. etc. etc.
1W 1.00 1.33 1.50 1.60 1.67 1 PIT 1 PEAK
2W 1.00 1.20 1.22 1.17 1.09 2 PITS 6 PEAKS
3W 1.00 1.07 0.96 0.82 0.68 2 PITS 4 PEAKS
MULTIPLE FAVORABLE FACTORS WITH INDEPENDENT UNFAVORABLE EFFECTS

FIGURE 5. Similar to figure 3 but unfavorable effects of the four factors are on distinct characters, subject to the independent homeostatic control.

loci there are adaptive pits at each extreme (*aabbccdd* and *ABCD*) and six adaptive peaks, corresponding to the combinations with any two dominant factors. The peaks are all at the same level in this case but this is because of the complete identity assumed for the effects of the four loci and would not be the case in general.

It is of interest to compare with this a situation which is the same except that the unfavorable tendencies of the four genes are each for a different character (Figure 5). It is evident that there is considerably less likelihood of multiple peaks since each of the unfavorable tendencies may be

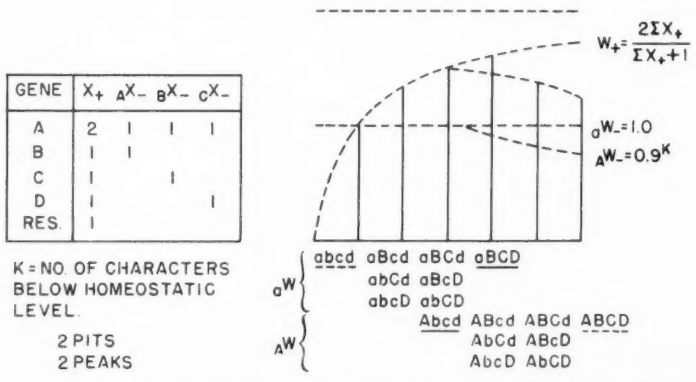


FIGURE 6. A major factor (*A*), three minor factors (*B*,*C*,*D*) and residual heredity (Res.) contribute favorably to one character subject to damping. Factor *A* has three independent unfavorable pleiotropic effects. Factors *B*, *C* and *D* have unfavorable pleiotropic effects each on one of those affected by *A*. Net selective value of populations allowing for damping of favorable effects and homeostatic control of unfavorable ones shows two widely separated peaks, *Abcd* and *aBCD*.

subject to homeostatic buffering, independently of the others. There is no necessity however that the unfavorable effects should be wholly prevented. Various numbers of peak genotypes may be present depending on the amounts of the various effects.

It may be added that the existence of multiple peaks is also not dependent on damping of the favorable effects of the genes, although more likely with such damping than without.

In actual cases it would be expected that some of the unfavorable pleiotropic effects would be on the same character (from the standpoint of homeostatic control) and others on different ones. Figure 6 is a model of such a case. With the chosen selective values there are two peaks, a lower one, *A- bb cc dd*, and a higher one, *aa B-C-D-*, separated by a wide and deep valley (four steps). In general, there would seem to be more likelihood of reaching a higher grade of an adaptive character by a combination of multiple minor factors with only minor and independent deleterious tendencies, if any, than by a major factor, more likely to have multiple serious deleterious tendencies which would tend to supplement those of other, otherwise favorable, factors.

TABLE 1

MACROMUTATION A INCREASES SELECTIVE VALUE 50% IN ONE RESPECT, REDUCES SELECTIVE VALUE 20% IN EACH OF 3 RESPECTS.

EACH OF GENES *B*, *C* AND *D*, IN PRESENCE OF *A*, RAISES SELECTIVE VALUE IN A DIFFERENT ONE OF ABOVE RESPECTS TO .90. EACH CAUSES 1% DECREASE INDEPENDENTLY OF *A*.

Genotype	W	Genotype	W
abcd	1.00	*Abcd*	.77
aBcd etc.	.99	*ABcd* etc.	.85
aBCd etc.	.98	*ABCd* etc.	.95
aBCD	.97	*ABCD*	1.06

A can become abundant only where *BCD* predominates.

The most probable way by which a major factor of the above sort might be utilized is by joint fixation of relatively specific modifiers of its unfavorable effects in spite of slight disadvantageous effects of the latter. (Table 1). This is an example of what Dobzhansky and Wallace (1950) call coadaptation. (cf. also Wallace, 1953.)

INCOMPATIBLE ADAPTATIONS

Another important sort of case in which more than one peak genotype is to be expected is that in which there are more or less incompatible ways by which the species may meet the challenge of the same environment. The case of complete incompatibility of two such adaptations is illustrated in figure 7. The situation represented is almost the opposite of the case in figure 4 in that there are two peaks at opposite extremes and six intermediate pits. If incompatibility is not complete, however, as illustrated in figure 8, there may be three peaks, the two modes of adaptation in pure

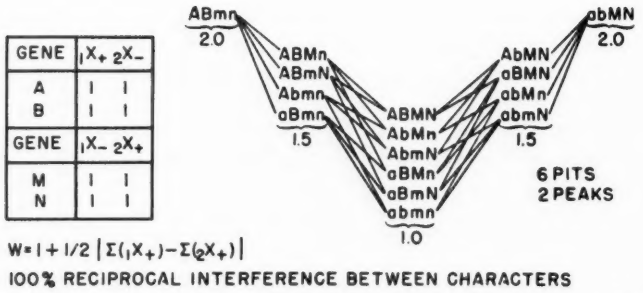

GENE | 1X+ 2X−
A | 1 1
B | 1 1
GENE | 1X− 2X+
M | 1 1
N | 1 1
ABmn 2.0
ABMn ABmN Abmn aBmn 1.5
ABMN AbMn AbmN aBMn aBmN abmn 1.0
AbMN aBMN abMn abmN 1.5
abMN 2.0
6 PITS 2 PEAKS
W=1+1/2 |Σ(1X+)−Σ(2X+)|
100% RECIPROCAL INTERFERENCE BETWEEN CHARACTERS

FIGURE 7. Genes *A* and *B* contribute toward one adaptation, *M* and *N* toward a completely incompatible alternative. There are two widely separated peaks in selective value (*ABmn*; *abMN*).

form *AB m m n n*, *aa bb MN* and a combination (*ABMN*) that may have a higher selective value but be inaccessible from the multiple recessive as the starting point.

Recent selection experiments of King indicate that resistance of Drosophila to DDT may be of this sort. Marked success in two different lines was maintained in F_1 but largely lost in F_2.

A situation similar to the last model occurs with respect to adaptation for slightly different ecological niches throughout the range of the species (different adaptive subzones of Simpson (1944)), on measuring mean selective value by weighting the selective values in the different niches by their prevalence.

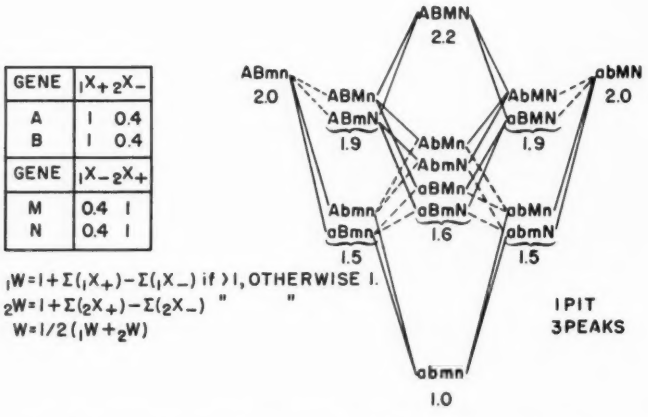


FIGURE 8. Similar to figure 7 but with only partial incompatibility. The highest peak of selective value (*ABMN*) is widely separated from two other peaks, *ABmn* and *abMN*.

The above models refer only to single favorable characters in each case. Actually the surface of mean selective values depends on all loci, considered simultaneously.

If the eight favorable characters that have been considered are assumed to evolve independently of each other, the net selective values are the products of the values indicated for each case. The total number of peaks is thus 8640 ($= 2\times5\times2\times6\times6\times2\times2\times3$) (taking the largest number in the fourth case). Twenty independent characters averaging 4 peaks each would give 10^{12} peaks. Actually, one would expect many more pleiotropic effects and extensive interlocking of characters.

One would also expect more heterallelism at peaks than indicated above in view of the accumulating evidence that overdominance is common. (East 1936, Crow 1948, Reeve and Robertson 1952, Dobzhansky and Wallace, 1953, Lerner 1953).

EFFECTS OF CHANGING ENVIRONMENT

It has been assumed in the preceding discussion that the conditions of selection are constant, apart from heterogeneity within the range, in the last case. In nature, the conditions are continually changing, sometimes only very slowly but sometimes rapidly. This means a continual shifting of the goal toward which mass selection is directed and in general a continual tendency toward loss of adaptation. It also means, however, that the species is kept continually on the move on the changing surface of selective values, instead of being bound by the mass selection to a single peak. The species is on a sort of treadmill but not a stationary one. It tends to work its way gradually toward the permanently higher regions in the surface. "Here we undoubtedly have," as noted in an earlier paper (Wright 1932), "an important evolutionary process and one that has been generally recognized."

It is fairly obvious, however, that even in this case, the process of continual readaptation would be more effective if it could be based on the adaptiveness of genotypes or systems of these, than merely on the momentary net effects of the separate genes. It is thus a question of primary importance in the theory both of evolution in a steady and in a changing environment, whether there is any way in which selection can be of the more effective sort referred to above.

ALTERNATION OF UNI- AND BI-PARENTAL REPRODUCTION

One possibility is a combination of prevailing uniparental reproduction with occasional crossbreeding. As already noted selection among clones is by the genotype as a whole and is an extremely effective process. If crossing occurs while there are still widely different genotypes on hand in the competing populations, recombination will provide an extensive new array of clones, all probably novel, but based on the successful ones of the preceding selection cycle. The field of variability is comparable to that under random mating but the binding of the population to a single adaptive peak

is avoided. There is the effective selection by genotype found in a heterogeneous array of clones but not the destruction of the store of variability and resulting stagnation that occurs under exclusive uniparental reproduction. The combination of the two systems has synergic effects (Wright 1931a).

This is a system that occurs in many organisms: viruses, bacteria, protozoa, trematodes, rotifers, cladocera, aphids, and many plants. It does not, however, predominate in the groups that have evolved the most complex adaptations: most higher plants, most arthopods, cephalapods and especially the vertebrates.

It is a system of violent alternations. The selection of favorable genotypes is followed by the breaking down of these by crossing, except in those respects in which all successful clones are alike, and in these respects no new field of variability is re-established by crossing. It is a system that is probably more significant as a means of insuring the appearance of adaptive genotypes, capable of taking advantage of any temporary bonanza, whatever the special conditions, than of progressive evolution.

INTERDEME SELECTION

A more effective way by which selection may be based on genotypes as wholes or (better) systems of such genotypes is by what I have called in early papers intergroup selection (Wright 1931a, b) but for which interdeme selection is perhaps a better term. The members of any reasonably large species do not actually interbreed at random. Most species occupy such extensive ranges in relation to the dispersal of individuals that random mating is impossible. Moreover, it is unusual to find the range occupied with anything like uniform density. The individuals are found in largely isolated communities where the local conditions are favorable. Dr. George Carter (1951) has stated "without doubt, the organization into communal populations is a general and fundamental fact of natural history. For this population, the term '*deme*' has been proposed" (Gilmour and Gregor 1939).

The demes are not completely isolated. Selection, with the deme as its unit, takes the form of more rapid growth of population of the better adapted ones and their more extensive dispersion into the territories of the less adapted with consequent grading up, or even replacement, of the latter.

Like any other mode of selection, interdeme selection can operate only if there is a source of variation among its objects that is independent of the course that ultimately emerges. In this case, the significant variability is differentiation of the demes. This variability can best be described in terms of the array of gene frequencies at all heterallelic loci. Selection is here according to *sets* of gene frequencies, the mean selective values of which depend on the communal adaptiveness, or coadaptation, of the whole set of genotypes, rather than on the net effects of genes or even of genotypes. Robertson and Reeve (1952), Dobzhansky and Wallace (1953), Lerner (1953), and others have recently stressed the importance of heterallelic systems in adaptation and in adaptive evolution.

The most obvious cause of differentiation of demes is, no doubt, adaptation to different local conditions by local mass selection. This process may sometimes lead to genetic complexes in one locality that have general value, but ordinarily this is not the case. The most important result is to give the species wide adaptability and a large store of variability. If differentiation is great enough there is splitting of the species into recognizable subspecies and even species. Splitting of species is a most important evolutionary phenomenon, but we are here considering transformation as a single species.

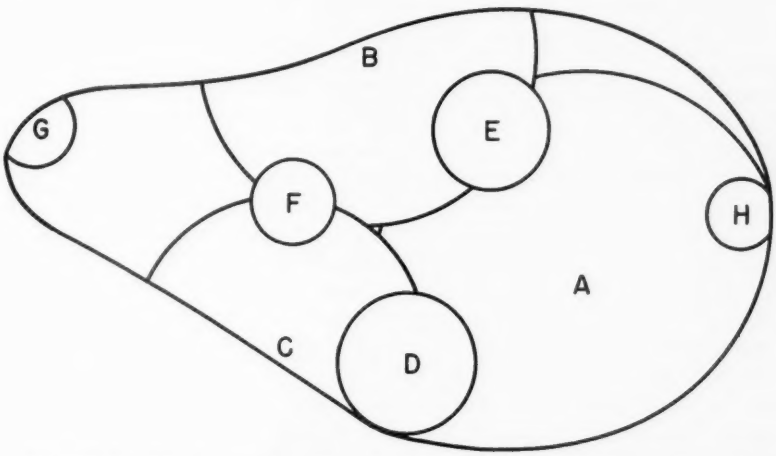


FIGURE 9. Interdeme selection within the range of a species, under uniform conditions. Relatively adaptive demes A, B, C, G arise from time to time from joint action of random processes and local mass selection. These expand, suffering modification in the process. New, more highly adaptive demes arise, especially in regions of contact (D,E,F) of the zones of influence of previous adaptive demes.

There are several ways in which random differentiation of demes may occur under the same prevailing conditions. Comparisons can be made by finding the distribution of gene frequencies at loci when specified rates of recurrent mutation, specified amounts of immigration and mean gene frequency of immigrants, specified mass selection coefficients (all of which tend to shift gene frequency toward a certain equilibrium point), are in statistical balance with the scattering due to random processes. Among the latter are the cumulative effects of accidents of sampling in small populations (Wright 1931a), fluctuations in the position of an intermediate optimum (Wright 1935) and fluctuations in the coefficients for the various directed processes referred to above. The nature in specific cases has been shown in a paper in 1948 on the basis of a general formula, presented earlier (Wright 1938).

The term random drift has often been applied solely to effects of accidents of sampling but should properly be applied to all random processes (in contrast with the steady drift from the directed processes) since as far as the theory of evolution by interdeme selection is concerned, the kind of random process that provides its raw material is largely irrelevant.

SOME MISUNDERSTANDINGS

There has been considerable misunderstanding of the role which I attribute to random drift. People like explanations in terms of single factors. Thus they like to think of characters as either wholly hereditary or wholly environmental. They tend to support one or another factor (mutation, hybridization, selection, etc.) as the essential factor of evolution and to classify theories of others similarly. If a certain change is shown to require a macromutation it is argued that the gradual accumulation of small differences is ruled out as a valid evolutionary phenomenon, or vice versa. If evidence is found for a selective difference between particular populations, this is held to prove that random processes have played no role in this, and again vice versa.

I have sometimes been labelled as an exponent of random drift as the only significant evolutionary phenomenon. When I deny this, a quotation from one of my early papers, (Wright 1931b), stated to apply to "non-adaptive radiation" is offered in evidence (Fisher and Ford 1950, Wright 1951). "In short, this seems from statistical considerations to be the only mechanism which offers an adequate basis for a continuous and progressive evolutionary process." This would seem to make my position in 1931 clear enough to the reader unless he is wary enough to go back and see what I actually wrote. If he does so, he will find that the two sentences preceding the quoted sentence were as follows: "The process of *intergroup selection* may be very rapid as compared with mass selection of individuals among whom favorable combinations are broken up by the reduction-fertilization mechanism in the next generation after formation. With partial isolation and differentiation, accompanying expansion of the successful subgroup, the process may go on indefinitely."

If my position in 1931 is to be labelled with a single word it would obviously have to be selectionist since "selection" is the noun antecedent to "this" in the quoted sentence, but this by itself would be highly misleading without adding that the mode of selection that seemed to me most effective is one that depends on the synergic effects of most of the other suggested factors of evolution.

In this connection, there is another misunderstanding that I would like to clear up if possible. The role that I have attributed to random drift in the process of interdeme selection is not in most cases that of bringing about *fixation* of one or another allele locally. Fixation may occur, but I have always held that "the end can only be extinction for a group permanently reduced below a certain size of population, in relation to other evolutionary factors" to quote from the same 1931 paper as above. What has been stressed has been *variation* of the gene frequencies as the condition at

loci most favorable for the process, recognizing that the amount of variation that is in a steady state will differ greatly at different loci in the same population in accordance especially with the severity of the local mass selection acting on each.

The reason is that fixation completely removes the locus from control by mass selection and leads to the extreme inbreeding effect referred to. Mere variation of gene frequency on the other hand implies a joint process in which mass selection prevents seriously deleterious inbreeding effect and can at any time amplify possibilities inherent in a chance momentary pattern of the gene frequencies at multiple loci, that happens to have been arrived at, while all are varying under the random processes. The difference is that between a frozen situation and a labile one.

SYNERGIC EFFECTS OF RANDOM PROCESSES

The synergic effects of different kinds of random processes has been illustrated by the case of long time fluctuations in the position of an intermediate optimum (Wright 1935). Selective value at two different times is plotted against the grade of a conventional multifactorial character in figure 10. Suppose that there is slow fluctuation between these situations throughout a species that is subdivided into many partially isolated demes. As already noted, mass selection in each of these leads toward approximate fixation of one of the peak genotypes (or at most heterallelism at one locus). If all of the demes start from near-fixation of the same peak genotype, a small lowering of the optimum, persisting for a long time, tends to bring about loss of one of the plus factors, but a different one in different cases according to slight differences in the frequencies of these (figure 11). On return for a long time to the higher optimum, there is approach in all cases to a peak genotype with the original number of plus factors, but which set again depends on small random differences in the gene frequencies among

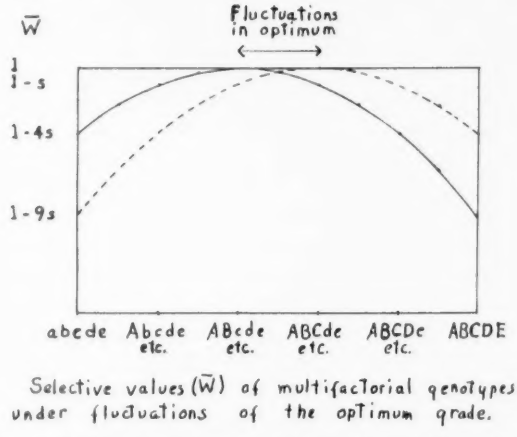


FIGURE 10. Selective values of the grades of a multifactorial character with intermediate optimum which shifts between two values.

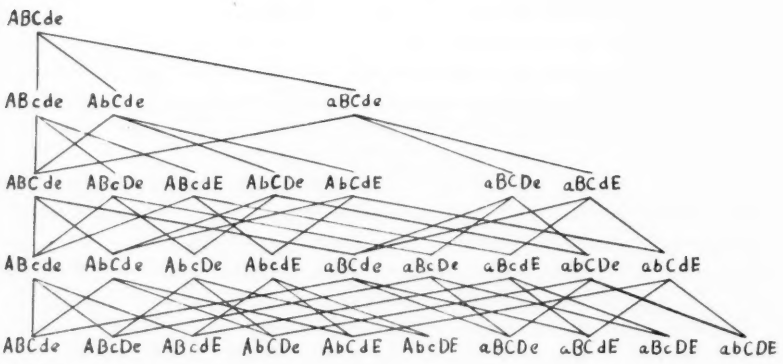

ABCde
ABcde AbCde aBCde
ABCde ABcDe ABcdE AbCDe AbCdE aBCDe aBCdE
ABcde AbCde AbcDe AbcdE aBCde aBcDe aBcdE abCDe abCdE
ABCde ABcDe ABcdE AbCDe AbCdE AbcDE aBCDe aBCdE aBcDE abCDE
Local differentiation from fluctuations in the optimum of a multifactorial character.

FIGURE 11. Array of possible predominant genotypes in a deme that may arise from random drift due jointly to a fluctuating optimum of a multifactorial character and accidents of sampling.

the demes. After a few such fluctuations, it is to be expected that all peak genotypes under each set of conditions will have approached fixation in some deme at some time. If there is one that has a considerable advantage because of pleiotropic effects the opportunity is given for effective interdeme selection and ultimate predominance of this genotype at least in one phase of the fluctuating conditions, and the best adapted at one remove in the other phase.

If selection proceeded to fixation in each phase, the accidents of mutation could determine which genotype would prevail in the next phase, but complete fixation under selection is an exceedingly slow process.

MACROMUTATIONS

There are probably, as Goldschmidt especially has urged (1940, 1952), important evolutionary changes in direction that rest primarily on single macromutations. There is no necessity to suppose that these become established by the abrupt origin of a new viable species by a concomitant isolating mechanism. Macromutations probably always bring about serious maladjustments and are strongly selected against, in spite of the occasional possibility of a major adaptation if the maladjustments can be smoothed out. Such mutations may be maintained at low but appreciable frequencies by recurrent mutation especially if the severity of selection is partially mitigated by low penetrance. It is exceedingly unlikely that random processes would ever bring about direct fixation. There is a reasonable chance, however, that random drift among the gene frequencies of *modifiers* may lead to a constellation in some deme at some time in which the sign of the selection coefficient of the macromutation becomes positive and it, along with its array of modifiers, becomes locally established and ultimately makes over the whole species by interdeme selection. (Wright 1951)

RECIPROCAL TRANSLOCATIONS

In cases in which there is enormous selection against a mutant heterozygote, but not against the mutant homozygote, the latter may be established by random drift if this drift also is enormous in amount. The most important case is that of a reciprocal translocation in a population with exclusive biparental reproduction. The selection against the translocation is enormous assuming the heterozygote to be semisterile as is usually the case. To pass the genetic barrier at aberration frequency 50 percent, there would have to be a comparably enormous random process. This can probably occur only if there are many localities in which colonies of the species are continually becoming extinct to be started anew from single gravid females or at least from a very small number of immigrants (Wright 1940, 1941). As Spurway (1953) has put it "The population size N of Wright should be so small that it may be profitable to think of *some* species originating from a single pair in a new Eden. Being an Adam or Eve gives a monster a chance to hope." She has pointed out that the translocations which distinguish sub-species of the newt, *Triturus cristatus*, in Europe can best be accounted for by supposing that the species reoccupied its range after the Pleistocene by successive crossings of hundreds of mountain passes by single sperm-carrying females, to establish colonies which at first, at least, came wholly from her offspring. The conditions for occasional fixation of a new chromosome pattern would be as great as possible.

INTROGRESSIVE HYBRIDIZATION

The expansion of successful demes into the territories of others, and the consequent formation of new genetic complexes which may tend to displace even the more successful parent deme, is somewhat like the introgressive hybridization of Anderson (1949) except that it is on a finer scale. Anderson stresses the aspect of new variability while I have stressed the selective aspect; but introgressive hybridization can be a significant evolutionary process only if demes that are successful arise from the hybrid swarm. In both interdeme selection and introgressive hybridization, linkage is significant in giving temporary coherence to certain gene complexes (cf. Wright 1931a, p. 146). The principal difference apart from the difference in coarseness is that interdeme selection can operate continuously since differentiation of demes may occur concomitantly with spread of the more successful ones, by means of the joint action of random drift and local mass selection on the variable material arising from the cross-breeding, while introgressive hybridization is presumably a relatively infrequent phenomenon dependent on previous differentiation of species by other processes.

SELECTION AMONG SPECIES AND HIGHER CATEGORIES

Finally, I will touch on the operation of selection in which the objects are at a still higher level than the demes. This is selection among non-interbreeding species and higher categories.

The raw material for such selection is even less random in an absolute sense than in the case of interdeme selection since it consists in the differentiation of these categories that has come about as a result of all of the preceding evolutionary processes. The competing groups may, however, be looked upon as random trials from the standpoint of the course of evolution of life as a whole that comes out of their competition.

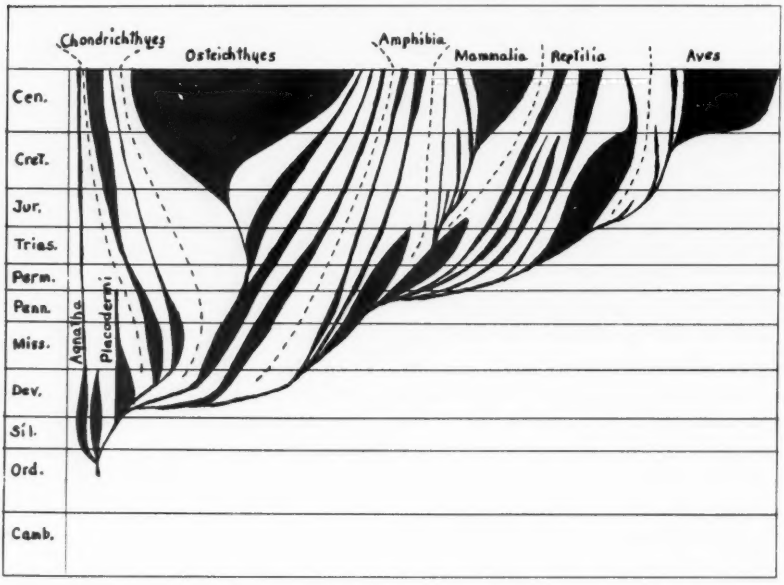


FIGURE 12. The history of vertebrate subclasses illustrating the extent to which the modern families trace to a very small number of forms at the beginning of the Mesozoic.

If there were no selection between such categories, we would expect to find all of those of an early geologic period persisting and all branching to similar extents. Nothing could be farther from the situation that is actually found. (cf. Figure 12 based on Romer 1945). There are some 40,000 species of vertebrates of very diverse types existing now. There was a somewhat comparable diversity and presumably thousands of species at the beginning of the mesozoic. But some 98 percent of the living families may well trace to only about eight of the species of the earlier period (a chondrichthyes ancestor of all sharks and rays, a holostian ancestor of all teleost fishes, a common ancestor of all urodeles and one of all anura, a cotylosaur ancestor (or two) of all turtles, an eosuchian ancestor of all lizards and snakes, a thecodont ancestor of all birds (and perhaps crocodiles), and a therapsid ancestor of all mammals (except perhaps the monotremes).

Probably only about two dozen of the species of vertebrates at the beginning of the mesozoic have left any descendants at all. The rich di-

versification of the modern vertebrates thus is based on a very limited number of successful types among the many that existed at the earlier period. The course of evolution of vertebrate life and of life in general has been guided throughout by a hierarchy of processes of selection ranging from selection between genes to selection between orders, classes and even phyla.

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Vol. XC, No. 850 The American Naturalist January–February, 1956

THE COMPOSITION OF AN OPEN POLLINATED SEGREGATING COTTON POPULATION

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PURPOSE OF STUDY

The seeds obtained from a field planting of Upland cotton result from a mixture of self and cross fertilization. The relative proportions of self and cross fertilization vary widely in different parts of the American cotton belt; the differences being associated principally with differences in the number and variety of pollen vectors available to effect pollination (Simpson, 1952). Simpson has suggested possibilities of utilizing natural crossing to conserve hybrid vigor by means of new cotton breeding methods and Stephens and Finkner (1953) have pointed out that it may even play a significant though not fully appreciated part in current breeding programs. In the latter paper, methods of estimating the proportion of natural crossing were reviewed and it was suggested that new methods would be necessary to obtain really critical information on the factors involved.

The amount of natural crossing which occurs in a mixture of genotypes under a system of open pollination is dependent not only on the number and activity of pollen vectors but also on the relative proportions in which the genotypes are represented in the common pollen pool. These in turn depend on the relative proportions of genotypes in the initial mixture and on their competitive efficiency. Competitive efficiency may reflect differences between genotypes in respect of numbers of flowers produced, number of pollen grains per flower, and a variety of physiological or mechanical features which might be expected to hinder or favor self-pollination. Very little is known about the relative magnitude of these variables, and in most estimates of natural crossing which have been made their effects have been confounded. It is of some theoretical and practical importance to attempt to devise methods of estimating separately the various factors involved.

The method of measuring natural crossing which has been usually adopted is to plant in isolation a mixture of two stocks, one carrying a seedling marker gene *R*, and the other its recessive allele, *r*. The proportion of *Rr* seedlings in the progenies from open pollinated *rr* plants is considered to be a measure of the amount of natural crossing which has occurred. This assumption should only be valid, of course, if a large excess of *RR* plants is present in the mixed planting, in which case intra-genotypic crossing (*rr* × *rr*) can be safely neglected and consequently the *rr* types in the open

Contribution from the North Carolina Agricultural Experiment Station. Published with the approval of the Director as Paper No. 634 of the Journal Series.

pollinated progeny can be considered to result from self-fertilization. If the proportion of *rr* types in the mixed planting is not negligible, approximate estimates of the amount of natural crossing can be obtained by appropriate weighting of the results obtained. However, the weighting should be based on the relative proportions of *R* and *r* pollen produced in the population (not on the relative proportions of *RR* and *rr* plants) and this can only be estimated very approximately (Finkner, 1954b).

A further disadvantage of the method is the fact that the estimates obtained are only strictly applicable to the stock mixture employed in the mixed planting. Differences between stocks in respect of time of anthesis, relative pollen abundance, relative pollen establishment etc., may bias the results obtained and make their general application of doubtful validity. By employing mixed planting of three stocks, differentiated by three contrasting seedling marker genes, Finkner (loc. cit.) showed clearly that stock differences of this type cannot be safely ignored. The estimates he obtained from the same mixed planting varied from 40% to 70% natural crossing depending on the stock combinations tested. He also obtained evidence of interaction between stock combination and locality in replicated experiments.

From the genetic point of view the apparent proportion of natural crossing in a mechanical mixture of stocks is dependent on the total genotypic differences between the stocks concerned insofar as these affect "crossability," and more specifically on pleiotropic effects of the seedling marker genes used to furnish the estimates on which it is based. In a mechanical mixture the "general" and "specific" effects are confounded and their relative importances cannot be evaluated. For this reason Stephens and Finkner (1953) suggested that estimates of natural crossing might be based more profitably on segregating populations, particularly in populations segregating concurrently for several seedling marker genes. In such material "general" genotypic differences should be segregating at random and hence the resulting differences should be neutralized. "Specific" differences associated with the seedling markers themselves could therefore be compared, pleiotropic effects analyzed, and estimates of the precision of the method obtained. An experiment of this type will be reported in this paper.

MATERIALS

The two parent stocks which provided material for analysis were contrasted in respect of four seedling marker genes as follows:

Parent A	*Parent B*
Red stem -*R*	Green stem -*r*
Non-yellow leaf -*V*	Yellow leaf -*v*
Normal leaf -*Cr*	Crinkle leaf -*cr*
Narrow leaf -*L*	Broad leaf -*l*

All Upland stocks which are grown commercially have the genotype, *rr, VV, CrCr, ll*. The parents listed above, therefore, include two dominant and two recessive mutants as compared with the "normal" type.

F_2 seeds obtained from a cross between the parent types were planted in the greenhouse in small paper cups. Over 95% germination was obtained. When the seedlings were well established they were transplanted to an isolation plot in the field. This was situated in the middle of a corn field surrounded by woodland on three sides and a tobacco field on the fourth side. It was therefore well isolated from extraneous sources of cotton pollen. The seedlings were planted in three rows, each row constituting a replicate in the subsequent analysis. A total of 663 seedlings was established, of which only two died early or failed to produce flowers.

All surviving plants were scored completely for the four seedling markers concerned. The proportions obtained were consistent with normal mendelian expectation except in the case of the *Cr* locus, where there was a significant deficiency in the recessive class (Table 1). When the experiment was begun it was thought that all the loci were independent but later it was discovered that *L* and *Cr* were loosely linked (circa 42% crossing over) and this fact has to be borne in mind in interpreting the results.

TABLE 1

SINGLE GENE RATIOS OBTAINED IN A F_2 POPULATION SEGREGATING FOR FOUR SEEDLING MARKERS

	Cr -	*crcr*	*R* -	*r r*	*L* -	*l l*	*V* -	*v v*
Freq.	531	130	515	146	480	181	506	155
χ^2	10.026		2.990		2.002		0.848	
P	.01-.001		.10-.05		.20-.10		.50-.30	

When the scoring was completed, plants having the following genotypes were selected as female parents for progeny analysis:

R -, *L* -, *cr cr*, *v v*
r r, *l l*, *Cr* -, *V* -
R -, *l l*, *Cr* -, *v v*
r r, *L* -, *cr cr*, *V* -
R -, *l l*, *cr cr*, *V* -
r r, *L* -, *Cr* -, *v v*

It will be noted that these six types represent every possible combination of doubly recessive genotypes. In order to obtain adequate sized progenies for testing it was necessary to pool the open pollinated seeds from plants with the same genotype. In each of the three rows the seeds from similar genotypes were pooled separately. In each case the pooled progeny was obtained from not less than three nor more than five plants. Progeny analysis was therefore based on a total of eighteen pooled progenies (3 rows with 6 genotypes per row). It was desirable to have some check on the accuracy of sampling, so triplicate samples of seeds from each pooled progeny were drawn and the seedling characters were scored separately for each sample. All seeds were acid-delinted and planted in small greenhouse beds.

METHODS OF ANALYSIS

Because the term "natural crossing" tends to be used rather loosely it will be convenient to introduce a more precise definition for use in these studies. Consider the six doubly recessive genotypes selected as female parents and listed in the previous section. These have the general genotypic formula, *aabb*. Their open-pollinated progeny will consist of four genotypes, *AaBb*, *Aabb*, *aaBb*, and *aabb*. The sum of the first three classes (excluding *aabb*) as a proportion of the total progeny represents the proportion of apparent out-pollination (AOP). The fourth class, *aabb*, results from a mixture of self and intra-genotypic pollination. The proportion of apparent out-pollination plus the proportion of intra-genotypic pollination represents the total proportion of out-pollination (TOP) which provides a measure of the total activity of the pollen vector and its contribution toward the setting of the crop. Providing the genetic markers employed were selectively neutral and that their frequencies in the F_2 population were strictly in accordance with mendelian expectation, one would expect that there would be no significant differences between the AOP estimates obtained from the six different genotypes used as female parents. Furthermore, all the classes included in the AOP estimates (*AaBb*, *Aabb*, *aaBb*) should be equal in size. Under these conditions an estimate of TOP could be obtained directly from AOP by simple weighting ($TOP = 4/3 \times AOP$). However, as shown in table 2, the proportions of the out-pollinated classes deviated from mendelian expectation, so that the estimation of TOP had to be calculated from the actual gene frequencies as will be shown later.

From these considerations it should be clear that any significant differences between the TOP estimates obtained from the six different genotypes used as female parents should be attributable to pleiotropic effects of the marker genes concerned on female crossability. Also any significant departures from expectation, based on gene frequencies, in the classes included in the AOP estimates should be due to pleiotropic effects of the markers concerned on pollen production and/or pollen efficiency.

In the experiments to be described it is assumed that the pollen vectors (chiefly bumble bees) work at random. Previous work at this station (Stephens and Finkner, 1953, Finkner, 1954a) is in accordance with this assumption. At the same time it is realized that a "normal" system of pollination would not necessarily hold for areas where the proportion of natural crossing is low or where different species of vector are active. It is possible, for instance, that where natural crossing is low, flower visitation by the vector might be more in accordance with a Poisson than a normal distribution. It is also possible that honey bees, which are important in some areas, might discriminate between different genotypes. A little consideration shows, however, that the latter situation would not be expected to introduce a bias—it would merely reduce the proportion of apparent out-pollination in *all* genotypes. In Raleigh, natural crossing is obviously extremely high, field observation shows that all flowers are repeatedly visited during the morning hours; and experimental data are available which suggests that

TABLE 2

SEGREGATIONS OBTAINED IN THE OPEN POLLINATED PROGENIES OF SIX DOUBLY RECESSIVE GENOTYPES SELECTED FROM AN ISOLATED F_2 POPULATION. EACH GENOTYPE IS REPLICATED IN THREE ROWS (SEE TEXT). THE FINAL COLUMN, AOP, IS THE APPARENT PROPORTION OF OUT-POLLINATION EXPRESSED IN PERCENT.

(A) Parent genotype: R-L- cr cr vv

Row	Cr V	Cr v	cr V	cr v	Total	AOP
1	27	57	17	33	134	75.37
2	33	35	15	21	104	79.81
3	19	37	17	57	130	56.15
	79	129	49	111	368	....

(B) Parent genotype: rr ll Cr-V-

Row	R L	R l	r L	r l	Total	AOP
1	21	24	51	130	226	42.48
2	17	35	38	148	238	37.82
3	20	52	23	175	270	35.19
	58	111	112	453	734	....

(C) Parent genotype: R-ll cr cr V-

Row	L Cr	L cr	l Cr	l cr	Total	AOP
1	41	31	57	78	207	62.32
2	39	24	40	25	128	80.47
3	12	11	13	14	50	72.00
	92	66	110	117	385	....

(D) Parent genotype: rr L-Cr-vv

Row	R V	R v	r V	r v	Total	AOP
1	9	7	8	37	61	39.34
2	32	25	25	118	200	41.00
3	13	15	25	97	150	35.33
	54	47	58	252	411	....

(E) Parent genotype: R-ll Cr- vv

Row	L V	L v	l V	l v	Total	AOP
1	34	34	29	97	194	50.00
2	25	23	36	79	163	51.53
3	24	22	43	113	202	44.06
	83	79	108	289	559	....

(F) Parent genotype: rr L- cr cr V-

Row	R Cr	R cr	r Cr	r cr	Total	AOP
1	15	12	30	28	85	67.06
2	40	21	65	67	193	65.28
3	28	17	100	101	246	58.94
	83	50	195	196	524	....

pollen applied to a cotton stigma two hours later than a prior excess pollination contributes to fertilization (Finkner, 1954c). This combination of circumstances might be expected to result in an effectively random system of pollination.

EXPERIMENTAL RESULTS

As explained earlier in this paper, the eighteen pooled progenies available for analysis were each divided into triplicate samples which were planted out in greenhouse beds in October, December and March successively. Contingency tests showed no significant heterogeneity between samples from the same pooled progeny except in the case of those progenies which were segregating for the Crinkle gene (*cr*). Further analysis showed the departure from homogeneity within these progenies was entirely attributable to a deficiency of Crinkle plants in the December planting, the segregation of other genes in the same progenies being homogeneous between samples. Previous experience with greenhouse plantings in the winter months has made it evident that under such conditions the penetrance of the Crinkle gene is much reduced, but it had not been anticipated that the reduction would be of such magnitude as to vitiate accurate scoring. However, since the heterogeneity between samples was confined to the December planting of those progenies which were segregating for Crinkle, it seemed permissable to exclude them from the experimental analysis and to pool all samples from the remaining progenies. Consequently, the segregations summarized in Table 2 represent the pooled data from triplicate samples in all progenies not segregating for Crinkle, (genotypes B, D and E) and the pooled data from *duplicate* samples in those progenies which have Crinkle segregates (genotypes A, C and F).

(1) *Apparent out-pollination* (AOP)

The first point of interest to be considered in table 2 is the apparent proportion of out-pollination (AOP) which is shown in the final column in each segregation. It is evident that the AOP estimate varies considerably according to the genotypic segregation on which it is based. In general the AOP estimate is high when determined from the open pollinated progenies of plants homozygous for Crinkle (genotypes A, C and F). Since the AOP

TABLE 3.

ANALYSIS OF VARIANCE OF THE AOP ESTIMATES GIVEN IN TABLE 2.

	D.F.	Sum. sqq.	Variance	"F"	
Rows	2	251.8902	125.9451	3.69	insig.
Genotypes	5	3524.9060	704.9812	20.66	<.001
Cr v cr 1		3217.3568	3217.3568	94.30	v. low
Residual 4		307.5492	76.8898	2.25	insig.
Error	10	341.1993	34.1199	....	
Total	17	4117.9955	....	....	

Mean apparent out-pollination

Genotypes:	*l cr*	*cr v*	*r cr*	*l v*	*r v*	*r l*	S.E.
	71.60	70.44	63.76	48.53	38.56	38.50	±3.37
		68.60			41.86		

estimates all lie within the range of 35 per cent to 80 per cent it is permissable to apply an analysis of variance to the percentage estimates without transforming the data. The results of this analysis are presented in table 3. The analysis shows that the AOP estimates determined from different rows in the experimental plot do not differ significantly, which may be taken to lend some support to Finkner's (1954a) finding that natural crossing under Raleigh conditions is effectively random. On the other hand the AOP estimates obtained from different genotypes show very large and highly significant differences. The inter-genotypic variance is further analysed into two parts (1) attributable to differences between *Cr*- and *cr*-containing genotypes and (2) a residual component which is a composite of all other genotypic differences under test. It is clear that the variation in AOP estimates is almost entirely due to the high proportion of apparent out-pollination obtained from Crinkle plants as compared with other genotypes. Crinkle genotypes on the average show an apparent out-pollination of 68.60 per cent as compared with 41.86 per cent for Non-Crinkle genotypes.

(2) *Composition of the pollen "pool"*

The second point of interest in table 2 is the marked deviation from equality which occurs in most of the out-pollinated classes (i.e. the classes listed in the first three columns in each segregation). These classes should occur in a 1:1:1 ratio if the gene frequencies in the original population were in accordance with mendelian expectation and if all genotypic combinations in the male gametes had equal viabilities. It will be noted that there seems to be a general tendency for those classes which contain double mutant combinations (*R L* in B, *L cr* in C, *R v* in D, *L V* in E and *R cr* in F) to be small. The double mutant class in A is the double recessive class, *cr v*, which presumably includes selfed and out-pollinated progeny, and hence cannot be compared with the other double mutant classes.

The out-pollinated classes in each row provide two estimates of the relative frequency of a normal gene and its mutant allele. Thus in table 2, Genotype A, Row 1, a comparison of the classes *Cr V* and *Cr v* gives a measure of the relative contribution of *V* and *v* pollen and, similarly, a comparison of classes *Cr V* and *cr V* measures the relative contribution of *Cr* and *cr* pollen. Altogether the data in table 2 provide nine estimates of the relative contributions of each allelic pair.

Contingency tests of the estimates so obtained were made to determine (a) if the four allelic pairs – *Cr*:*cr*, *R*:*r*, *L*:*l* and *V*:*v* occurred in different ratios (b) if ratios obtained for each allelic pair were influenced by the family or row from which they were obtained. The results of these tests are summarized in table 4. In this table the total heterogeneity is first partitioned into a portion due to differences between allelic pairs with 3 degrees of freedom and a portion within allelic pairs with $4 \times 8 = 32$ degrees of freedom. Both items show considerable heterogeneity, but as shown in the table, if the mean squares obtained from the χ^2 values are compared in an "F" test, the "between pair" effects are much greater than the "within

pair" effects. It may be concluded that most of the variation is due to the fact that the four allelic pairs occur in different ratios. The pooled data for each of the four ratios is shown at the foot of table 4.

The heterogeneity "within pairs" can then be partitioned for each allelic pair separately into 3 components ascribable to differences between (a) families (b) rows and (c) family × row interactions. This partitioning shows that the ratios of two of the allelic pairs, *Cr*: *cr* and *L*: *l*, are homogeneous. *R*: *r* ratios are heterogeneous; apparently showing significant differences

TABLE 4.

CONTINGENCY TESTS OF RATIOS OBTAINED IN THE OUTPOLLINATED CLASSES SHOWN IN TABLE 2.

Source of heterogeneity	D.F.	χ^2	Mean Square	F
Between allelic pairs	3	67.73***	22.8	11.64***
Within allelic pairs				
(a) *Cr*: *cr*				
Families	2	0.64	0.32	1.07
Rows	2	2.52	1.26	4.20
Fams × rows	4	1.21	0.30	....
(b) *R*: *r*				
Families	2	11.90**	5.95	2.56
Rows	2	6.69*	3.35	1.44
Fams × rows	4	9.26	2.32	....
(c) L: l				
Families	2	5.25	2.63	2.63
Rows	2	5.89	2.95	2.95
Fams × rows	4	3.99	1.00	....
(d) *V*: *v*				
Families	2	9.44**	4.72	15.73*
Rows	2	4.23	2.12	7.06*
Fams × rows	4	1.21	0.30	....
Total	32	62.23***	1.94	
Grand total	35	129.96***		

*significant at 5% level; ** at 1% level; *** at 0.1% level.

	Cr	*cr*	*R*	*r*	*L*	*l*	*V*	*v*
Pooled data for each allelic pair	254	165	195	365	233	329	216	255
Mutant class as percent of total	39.38		34.82		41.46		54.14	

between families and between rows. However, when these components are compared with the family × row interaction component in an "F" test, the differences are not significant. This means that although the *R*: *r* ratios show considerable variation from sample to sample this variation cannot be ascribed with confidence to differences between families nor differences between rows *per se*. In the case of the *V*: *v* ratios, the appropriate χ^2 and "F" tests show that there are significant differences between families and

that both family and row effects are significantly greater than effects due to family × row interaction.

Considering the data as a whole it may be concluded that the alleles in each pair occur with very different relative frequencies, and that although the ratio of a gene to its mutant allele is more variable than can be ascribed to chance sampling error, such variation is relatively low in magnitude and not consistently associated with family or row differences.

The combined data from each inter-allelic comparison show that there are large deviations from equality except in the case of the *V*:*v* comparison. in the other three comparisons the mutant classes *cr*, *R* and *L* are markedly smaller than the corresponding normal classes. Three factors might con-

TABLE 5.

COMPARISON OF OBSERVED GENE FREQUENCIES GIVEN IN TABLE 4 WITH THOSE EXPECTED FROM THE COMPOSITION OF THE F^2 POPULATION (CALCULATED FROM DATA IN TABLE 1).

	Cr	*cr*	*R*	*r*	*L*	*l*	*V*	*v*
Obs.	254	165	195	365	233	329	216	255
Exp.	233	186	297	263	268	294	244	227
χ^2	4.264		74.589		8.738		6.667	
P	.05-.02		<.001		.01-.001		.01-.001	

tribute to these deviations, (1) unequal frequencies in the initial F_2 population (2) unequal production of pollen by different genotypes (3) selective advantages of normal over mutant pollen. The contribution of the first factor can be estimated by comparing the expected gene frequencies in the F_2 population with their observed frequencies in the out-pollinated classes in table 4. The expected frequencies can be estimated from the F_2 segregations shown in table 1 where it can be seen that one of the segregations (*Cr*:*cr*) differs markedly from mendelian expectation. If all the genotypes in the initial F_2 population contributed equally to the common pool of pollen then the expected gene frequencies could be calculated in the following manner: From the data given in table 1 the proportion of *rr* genotypes is 146/661 or 0.2208775. The frequency of *r* pollen in the population is thus expected to be the square root of this value or 0.470 and the frequency of *R* will be 1 − 0.470 or 0.530. The expected frequencies of the other three allelic pairs can be calculated similarly. The actual gene frequencies in table 4 can then be fitted to the calculated frequencies as shown in table 5. Agreement between the two sets of data is very poor. Three of the four mutant genes, *cr*, *L* and *R*, appear less frequently in the pollen pool than would have been expected from the composition of the F_2 population. On the other hand, the mutant gene, *v*, appears more frequently than expected. Clearly the composition of the initial population of plants gives little indication of the relative composition of the pollen which it produces.

The second factor which might be important in determining the composition of the pollen pool is the relative production of pollen by different genotypes.

If mutant types produced less flowers or less pollen per flower than their normal sibs, one would expect a deficiency of mutant genes in the pollen pool. Some independent evidence obtained by Mr. J. C. Williams at this station (data unpublished) is of interest in this connection. He finds that the open pollinated progeny from a population segregating for the same *L* and *R* mutants shows a marked shift in frequency in favor of their normal alleles. Again, unpublished data of Mr. S. Cain at College Station, Texas, showed that the mutant segregates, *LL* and *RR* from the F_2 population yielded fewer bolls (and hence fewer flowers) per plant than their normal sibs. In a competitive system, therefore, shifts in favor of the normal genotype are to be expected and it is surprising that in the present experiment the frequency of the *v* mutant is shifted in the opposite direction.

The third factor which requires consideration is pollen competition. If this were an important factor selective differentials would be established between pollens of different genotypes, and the gene frequencies would vary accordingly. The four mutants used in this experiment have all been studied repeatedly in test crosses by several workers and no consistent deviations from expected mendelian ratios have been reported. If pollen competition were important one might expect that the segregations obtained would follow no consistent pattern—in fact the establishment of a mendelian ratio is good presumptive evidence that pollen competition is an unimportant factor in so far as the genes tested are concerned. For this reason it is not believed that the heterogeneity "within allelic pairs" in table 4 can be attributed to pollen competition. A more likely interpretation is that the quantity of pollen produced depends on the genotypic composition of the plant on which it is borne. Thus although pollen vectors might work at random from row to row through the experimental plot, and as a consequence the AOP estimates would not show significant row differences (table 3), yet the precise composition of the outpollinated classes might reflect local variations in the genotypic compositions of plants in the neighborhood of those sampled. Such variations however are not consistent, nor do they play a major role in determining the average composition of the open-pollinated population.

(3) *Total out-pollination (TOP)*

The genetic composition of the common pollen pool will naturally affect the AOP estimates obtained. A genotype which contributes relatively less to the pool should have less opportunity for intra-genotypic crossing and consequently more of the out-pollination it receives will be from "foreign" genotypes. In order to arrive at a useful estimate of total out-pollination it is necessary to eliminate the effects of different gene frequencies. In a random system a change in gene frequency leading to an increased amount of foreign pollination (AOP) should be exactly balanced by a decreased amount of intra-genotypic pollination, so that the total out-pollination which includes both, should remain constant.

The procedure used to estimate total out-pollination (TOP) is to calculate from the actual gene frequencies the expected amount of intra-genotypic

pollination and to adjust the AOP estimates accordingly. Consider the out-pollination of Genotype A in table 2. The single gene ratios are *Cr*: *cr* and *V*: *v*. The best estimates of the relative proportions of these alleles in the population are the mean percentages given in table 4. These are 39.38 per cent for *cr* and 54.14 per cent for *v*. Gametes carrying the combination *cr v* are therefore expected to occur with a frequency equal to the product of the individual gene frequencies (i.e. 39.38% × 54.14%, or 21.32%). The out-pollinated classes, *Cr V*, *Cr v* and *cr V* should therefore account for (100 − 21.32%) of the total out-pollination or 78.68 per cent. It follows that the AOP estimate for Genotype *cr v* of 70.44% which is given in table 3 only measures 78.68 per cent of the total out-pollination. Total out-pollination (TOP) should therefore amount to $\frac{100 \times 70.44}{78.68}$ or 89.53 per cent. TOP

TABLE 6.

ESTIMATED TOTAL PROPORTION OF OUT-POLLINATION (TOP) IN PERCENT FOR THE SIX GENOTYPES LISTED IN TABLE 2.

Genotypes:	*l cr*	*cr v*	*r cr*	*l v*	*r v*	*r l*
TOP %	97.12	89.53	85.78	71.04	59.59	62.26
		90.81			64.30	

estimates for all six genotypes can be calculated in similar fashion except that the estimate for *l cr* has to be weighted slightly to allow for the coupling linkage between *l* and *cr*. The TOP estimates so obtained are given in table 6. Their values indicate that most of the seeds set on genotypes homozygous for Crinkle can be attributed to out-pollination, the average estimate for the three Crinkle genotypes being over 90 per cent. On the contrary the Non-Crinkle genotypes give an average estimate of about 64 per cent.

(4) *The relation between foreign pollination (AOP), intra-genotypic pollination and selfing.*

The compositions of the open pollinated progenies of the six genotypes under test are summarized in table 7. In this table the frequencies of the same genotypes in the common pollen pool (calculated from the data in table 4) are presented for comparison.

The data show, as expected, that there is a strong negative correlation between the AOP and selfed fractions associated with the different genotypes. A more surprising feature is that the intra-genotypic fraction tends to remain constant over all genotypes. The reason for this situation can be best appreciated from a general theoretical consideration. For any given genotype, let *s* be the proportion of self-pollination it receives, and let *f* be its relative pollen output (i.e. the proportion by which it is represented in the common pollen pool). The expected composition of its open pollinated progeny will then be as shown in the rightmost column of table 7. The

TABLE 7.

SUMMARY OF PERCENTAGE COMPOSITION OF THE PROGENIES OBTAINED BY OPEN POLLINATION OF THE SIX GENOTYPES. FREQUENCIES OF THE SAME GENOTYPES IN THE POLLEN POOL ARE INCLUDED FOR COMPARISON.

Genotypes	*l cr*	*cr v*	*r cr*	*l v*	*r v*	*r l*	General
AOP	71.60	70.44	63.76	48.53	38.56	38.50	(1-s) (1-f)
Intra-genotypic	25.52	19.09	22.02	22.51	21.03	23.76	(1-s)f
Selfed	2.88	10.47	14.22	28.96	40.41	37.74	s
Total	100.00	100.00	100.00	100.00	100.00	100.00	1
Frequency in pollen pool	26.27*	21.32	25.67	31.69	35.29	38.16	f

*Adjusted for coupling between *L* and *Cr*.

expected proportion resulting from intra-genotypic pollination is $(1 - s)\ f$: a value which would obviously tend to remain constant if s and f were positively correlated. There is in fact a significant correlation between the actual s and f values in table 7 ($r = +0.8966$; $P = .02$-$.01$).

A rather simple explanation can be offered for this correlation. The relative pollen output (f) of a genotype should depend primarily on the number of flowers produced and the number of pollen grains per flower. The relative amount of selfing (s) should be unaffected by the number of flowers produced, but one would expect that a flower which produced abundant pollen would be self-pollinated to a greater degree than a flower which produced relatively little pollen. Both s and f therefore should be dependent on a common variable—the amount of pollen produced per flower. The genotypes which produce the largest amount of pollen per flower should self most, contribute most to the pollen pool and receive the least amount of foreign pollen. Intra-genotypic pollination should remain relatively unaffected. Reference to table 7 shows that most of the differences between genotypes can be interpreted satisfactorily on this basis. On the other hand, if variation in number of flowers produced were the more important factor in determining the compositions of the progenies from the different genotypes, the correlation between s and f should be obscured, and the intra-genotypic fraction instead of remaining constant should vary inversely with the AOP fraction. The data in table 7 lend no support to this alternative interpretation.

DISCUSSION

The analysis of natural crossing in a segregating population makes possible the separate estimation of some of its components.

(1) *Vector activity*

The estimates of total out-pollination (TOP) given in table 6 are independent of relative gene frequencies in the population and thus they provide an unbiased estimate of the total vector activity under the conditions of the experiment. Over half of the seeds set in all four genotypes tested resulted from out-pollination, the actual estimates ranging from 59.59% to 97.12%. In

general, genotypes homozygous for Crinkle showed the highest percentage of out-pollination.

Estimates of total out-pollination in Non-Crinkle types average 64.30 per cent, a figure which agrees reasonably well with those obtained earlier by Finkner (1954b). He used mechanical mixtures of stocks in a randomized system, but attempted to adjust his estimates in accordance with the expected composition of the pollen population. His estimates (in Raleigh) for out-pollination in his Virescent Yellow (*v*) stock were of the order of 67% which may be compared with 71.04% (*l v*) and 59.59% (*r v*) in table 6. Estimates for his Green Leaf stock averaged 45%, which may be compared with 62.26% for *r l* in table 6. The discrepancy here can most likely be attributed to the fact that the design of Finkner's experiment only allowed him to estimate out-crossing of his Green Leaf stock with *R* pollen. The relatively low production of *R* pollen as shown in table 4 would be expected to give an underestimate in his experiment.

On the whole these data suggest that as much as sixty per cent of the seed set by a cotton population in the Raleigh area may result from out-pollination by the local vectors (chiefly bumble bees). If this situation is typical for commercial plantings of cotton in this area one would suspect that the activity of the vectors may play an important part in the determination of yield. It has been commonly observed that the yield of cotton from self-pollinated bolls is less than from open-pollinated bolls. The difference has usually been attributed to the deleterious effect of bagging or other mechanical devices used in selfing. The question may perhaps be re-opened as to whether the potential yield of a cotton plant is practically attainable in the absence of a thriving population of pollen vectors. Some data obtained by Loden and co-workers (1950) for another purpose are of interest in this connection. They found that pre-pollination of unreceptive stigmas, followed by a second application of pollen at the normal time, gave an increase in seed-set of about 30% as compared with their controls. Three controls were used—"normally" selfed, hand-crossed, and open-pollinated flowers—and no significant differences between them were found. These experiments were carried out in Texas where natural crossing is very low. An obvious corollary of their results is the conclusion that if the pre-pollination technique was capable of increasing seed-set by this order of magnitude, then none of the control methods could have approached achieving the full seed setting capacity of their plants. It would seem that selfing, crossing and exposure to a low population of vectors might be equally inefficient in producing a full seed-set, as compared with the accomplishments of an active and adequately sized population of bumble bees.

(2) *The effective gene frequencies in the pollen pool*

The amount of hybridization which occurs in an open pollinated cotton population is dependent not only on the vector activity but also on the effective gene frequencies in the pollen pool. The best estimates of effective gene frequencies are the mean gene frequencies in the out-pollinated classes which are shown in table 4.

The effective gene frequencies are in turn dependent on four possible factors:

(a) The relative proportions of genotypes in the initial plant population.
(b) The relative number of flowers produced by each genotype.
(c) The relative number of pollen grains per flower.
(d) The competitive efficiency of pollens with different genetic constitutions.

The evidence which has been presented shows that the factors of chief importance are (a) and (c) above. The effective gene frequencies in the pollen pool are not directly proportional to the relative composition of the initial plant population, because different genotypes contribute unequally to the common pollen pool. The substitution of a single normal gene by its mutant allele is sufficient to alter the pollen output considerably. Three of the mutants tested, *cr*, *R* and *L*, reduced pollen output: the fourth, *v*, increased it. In general one would expect mutant "markers" to depress yield. An important component of yield is the number of flowers produced, and one might expect that reduced pollen output in mutants would be determined primarily by this factor. However the composition of the progenies resulting from open pollination in this experiment (table 7) suggests that the amount of pollen produced per flower may be more important than the number of flowers produced per genotype. Differences between genotypes in respect of the amount of pollen produced per flower would account for the positive correlation between relative pollen output and degree of selfing, the constancy of the proportion of intra-genotypic pollination, and the negative correlation between the proportion of foreign pollination (AOP) and selfing.

There was no indication that the genetic constitution of the pollen affects its competitive efficiency. Previous work has indicated that the markers used segregated in normal mendelian ratios, and in the present experiment their relative frequencies in the open pollinated progenies were not consistently affected by the genetic composition of the families in which they were recovered (table 4).

The general picture which emerges from these studies is that of a randomly operating population of vectors whose effects on the genetic composition of the plant population in which they work are largely determined by the mechanical interaction of three factors, (1) the number of vectors (2) the relative proportions of genotypes (3) the number of pollen grains produced per flower. While it is certain that this picture is oversimplified and subject to modification by other variables (e.g. variations in numbers of flowers per genotype, in time of flowering and possible in flower structure it is probably representative of the actual situation in a segregating population, where many of the minor factors would be distributed at random.

SUMMARY

(1) By analysing the open pollinated progeny from a segregating population of cotton whose initial composition in respect of four marker genes is known,

it has been possible to estimate separately the relative frequency of three types of pollination:

(a) The proportion of apparent out-pollination (AOP), i.e. "foreign" pollination.

(b) The proportion of intra-genotypic pollination, i.e. involving different flowers of the same genotype.

(c) The proportion of self-pollination (i.e. within the same flower). The sum of (a) and (b) is the total out-pollination (TOP). It is independent of the gene frequencies in the population and therefore provides unbiased estimates of the total vector activity.

(2) The amount of hybridization which occurs depends not only on the number and activity of the vectors, but also on the effective gene frequency in the population. The latter is shown to depend primarily on (a) the relative frequencies of genotypes in the initial population (b) the relative amount of pollen produced *per flower*.

(3) The amount of pollen per flower apparently is of major importance not only in determining the amount of selfing but also the amount of pollen contributed to the pollen pool, because these two estimates, as determined from the data, are highly correlated. As a result of this (positive) correlation it is shown that the proportion of intra-genotypic pollination remains relatively constant for all genotypes.

(4) Under the conditions of the experiment vector activity was extraordinarily high, resulting in total out-pollination estimates of 60 to 90 per cent, depending on the genotype involved. It is suggested that vector activity of this magnitude may be an important though overlooked factor in determining the yield of the crop.

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Vol. XC, No. 850 The American Naturalist January–February, 1956

CROSSING OVER AND INVERSIONS IN COADAPTED SYSTEMS*

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Wallace (1953) has recently proposed that combinations of certain chromosomal inversions will be rare in natural populations of *Drosophila pseudoobscura*; namely, combinations of simple inversions between which crossing over could occur. Such combinations of gene arrangements, taken in groups of three, have been termed *triads* by Wallace. Crossing over between these inversions is suggested as a means whereby the integrity of favorable or so-called coadapted gene combinations would be lost. For example, in populations of *D. pseudoobscura* the inversions known as Arrowhead (AR) and Pikes Peak (PP) would form a triad in combination with the Standard (ST) gene arrangement.

Since both AR and PP are simple inversions with respect to ST it is assumed that sufficient crossing over would occur between each of them and ST so as to permit the transfer of genes between all three chromosome types. Thus, Wallace states that double crossovers between ST and PP would prevent the maintenance of coadapted systems. On the other hand a combination of ST, AR, and CH (Chiricahua) is not an unexpected one in populations of this species since CH is a complex, overlapping inversion with respect to both ST and AR. Figure 1 represents the gene arrangements of AR, CH, and PP with respect to ST.

Wallace's hypothesis is based upon the fact, discovered in *D. melanogaster* by Sturtevant (1919), that crossing over is largely suppressed in inversion heterozygotes. Dobzhansky and Epling (1948), studying the same phenomenon in *D. pseudoobscura*, observed that crossing over was more highly suppressed among inversion heterozygotes involving complex, overlapping gene arrangements such as CH in combination with ST, than in the case of the simple AR inversion when in combination with ST. Thus, they concluded that inversion is a means whereby combinations of genes imparting a favorable adaptive character to their carriers can be retained without loss through recombination due to crossing over. This conclusion and the triad hypothesis of Wallace assumes logically that the complex inversion heterozygotes, because of conditions of their pairing, will show a suppression of crossing over greater than that exhibited by the simple inversions. The triad hypothesis depends to a large extent upon the assumption that a direct relationship exists between the cytological configuration of pairing in the salivary gland chromosomes of inversion heterozygotes and the genetic consequences of crossing over. The recent observations

*Supported in part by an institutional grant of the American Cancer Society and by a grant (RG-4000) from the National Institutes of Health, Public Health.

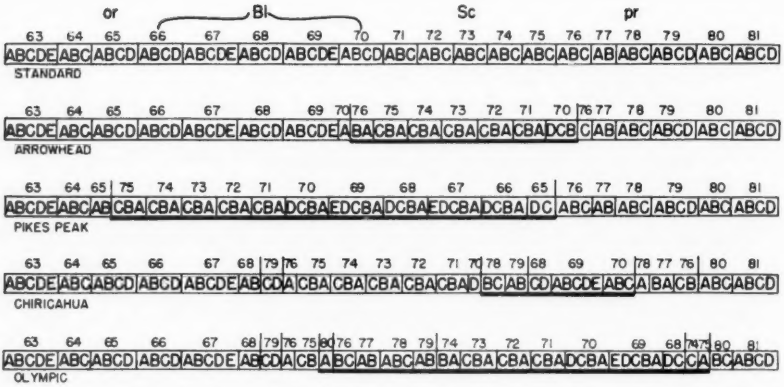

FIGURE 1. The Standard, Arrowhead, Pikes Peak, Chiricahua, and Olympic third chromosome gene arrangements in *Drosophila pseudoobscura*. (from Dobzhansky and Epling, 1944). The positions of the mutant loci *or*, *Bl*, *Sc*, and *pr* as determined by Tan (1937).)

of Dobzhansky (1952) and Epling (unpublished) that the frequency of the PP gene arrangement has increased significantly in certain populations of *D. pseudoobscura* are of interest in this regard. The populations studied by Dobzhansky and Epling are characterized by a relatively high frequency of both the ST and AR gene arrangements. The observation of increasing PP frequency is an unexpected one in terms of the hypothesis proposed by Wallace. In addition a new simple inversion has been found in San Jacinto populations of *D. pseudoobscura* (Epling, unpublished) which forms a triad with ST and AR. In view of these observations, it is therefore highly desirable to obtain genetic tests of the triad hypothesis. Accordingly, this paper presents data derived from the genetic analysis of crossing over in a variety of *D. pseudoobscura* third chromosome inversions as they relate to Wallace's triad hypothesis.

MATERIALS AND METHODS

Only the Standard (ST) third chromosome gene arrangement bears the markers suitable for crossover analyses; these are orange eye (*or*), Blade wings (*Bl*), Scute bristles (*Sc*), and purple eye (*pr*). The location of these markers relative to different inversions is given in figure 1. Crossing over has been studied between the marked ST chromosome and the wild type third chromosome gene arrangements known as AR, CH, OL (Olympic), PP, and VF (Vandeventer Flat). These inversions, with the exception of VF, are described in Dobzhansky and Epling (1944). VF is the arrangement recently discovered by Epling; it will be described shortly by him. It is to be noted that AR, PP, and VF are simple inversions with respect to ST while CH and OL are complex, overlapping inversions.

The experimental procedures followed in this study are identical with previously described analyses of crossing over in *D. pseudoobscura* as carried out in this laboratory (Levine and Levine 1954, 1955).

TABLE 1

CROSSING OVER BETWEEN THE *or Bl Sc pr* (ST) CHROMOSOME AND VARIOUS WILD TYPE THIRD CHROMOSOMES

		ST	*AR*	*PP*	*VF*	*CH*	*OL*
0	+	997	2136	2877	1082	2576	682
	or Bl Sc pr	622	1870	3227	1404	1774	848
1	*or*	117	54	0	224	22	2
	Bl Sc pr	118	70	0	204	18	6
2	*or Bl*	338	20	0	273	0	0
	Sc pr	254	16	0	237	3	0
3	*or Bl Sc*	444	0	0	32	0	0
	pr	362	10	0	27	2	0
1-2	*Bl*	6	0	0	4	0	0
	or Sc pr	5	1	0	1	0	0
1-3	*Bl Sc*	69	0	1	2	0	0
	or pr	42	0	0	0	0	0
2-3	*Sc*	88	0	0	2	0	0
	or Bl pr	51	2	0	0	0	1
1-2-3	*or Sc*	0	0	0	0	0	0
	Bl pr	0	0	0	0	0	0
Total		3513	4170	6105	3492	4395	1539

TABLE 2

PER CENT CROSSING OVER IN THE *or Bl Sc pr* REGION OF THE THIRD CHROMOSOME

	ST	AR	PP	VF	CH	OL	TL*
or-Bl	10.16	2.99	0.016	12.46	0.91	0.52	0.2
Bl-Sc	21.12	0.93	0	14.81	0.06	0.06	0
Sc-pr	30.06	0.23	0.016	1.80	0.04	0.06	0

*Data of Dobzhansky and Epling (1948).

RESULTS

The data are summarized in tables 1 and 2. It must be pointed out that there is significant variation in crossing over between different strains of ST and AR. The nature of this variation is discussed elsewhere (Levine and Levine 1955). From tables 1 and 2 it will be seen that crossing over in the complex, overlapping inversions (CH, OL, and TL) is highly suppressed while the simple inversions (AR, PP, and VF) have various effects on crossing over. It is seen that, compared with ST, VF has suppressed crossing over in the *Sc-pr* region only. The AR inversion shows suppression over the three regions while only one crossover out of 6105 flies was observed with the PP chromosome.

DISCUSSION

The results of the experiment reported here as regards the PP gene arrangement are of particular interest. They reveal, by virtue of almost com-

plete suppression of crossing over in the ST/PP heterozygote, a basis for the possible maintenance of an inversion system involving ST, AR, and PP. Thus, the fact that PP occurs frequently in populations in which ST and AR are also frequent is not in opposition to Wallace's hypothesis. However, this has been ascertained only by direct genetic tests of crossing over in the ST/PP heterozygote. Wallace's hypothesis is based on the effect of inversions on crossing over, but it has been shown here that these effects cannot always be predicted from cytological evidence alone. It would seem necessary, therefore, to characterize inversion types by their effect on crossing over in any discussion of their role in a coadapted system.

Of perhaps more general interest is the degree to which crossing over is suppressed in the ST/PP heterozygote. It is clear from the data presented in tables 1 and 2 that a number of crossovers are recovered even in the complex, overlapping inversions in heterozygous condition with ST. A few crossovers are expected on the basis of the mechanism described by Sturtevant and Beadle (1936) in which the only crossover types recovered are those which carry no duplications or deletions. It can be shown, however, that double crossovers are expected among the types recoverable from a relatively long simple inversion such as PP when heterozygous with ST (Sturtevant and Beadle, 1936). These crossovers are possible since they result in the production of normally constituted chromatids. Therefore, the double crossover classes as given in table 1 for regions 1-2, 1-3, and 2-3 were to be expected. The absence of observed crossing over, except for one crossover, might possibly result from the location of markers used with relation to the points of inversion. However, since both *Bl* and *Sc* lie within the inversion, double crossovers should have been recovered. Therefore the nature of the suppression of crossing over between the ST and PP chromosomes must be investigated further. It is clear, however, that it is unwise to draw inferences regarding crossing over in inversion heterozygotes from the cytological configurations of pairing as given by salivary gland chromosomes alone.

An important test of the triad hypothesis results from the presence of the new, simple VF inversion in the San Jacinto Mountain populations. Since there is almost free recombination between VF and ST, a stabilized coadapted system with ST is not predicted by Wallace's hypothesis. Data on the frequencies of this new gene arrangement in the San Jacinto populations which will bear upon the hypothesis are being gathered by Epling (personal communication).

Of the remaining inversions tested it may be said that except for AR, they show as expected an almost complete suppression of crossing over with ST. Thus, according to Wallace's hypothesis and the crossover data, stabilized systems of these inversions with ST are possible.

The data presented here show that crossing over in simple third chromosome inversion heterozygotes of *D. pseudoobscura* may range from being completely suppressed as was the case with ST/PP to being almost normal as shown by the ST/VF heterozygote. The complex, overlapping inver-

sions, however, behave as expected in that they show almost complete crossover suppression. Coupled with these differences in crossover suppression are the interchromosomal effects of these inversions (Levine and Levine 1954) and the variation in crossing over between different strains of the same gene arrangement (Levine and Levine 1955). These phenomena indicate the intricate nature of gene recombination in an inversion system and suggest the need for an evaluation of the role of recombination in the genetics of natural populations.

SUMMARY

Crossing over between the ST gene arrangement of *D. pseudoobscura* and various third chromosome inversions has been analyzed and considered in light of the triad hypothesis of Wallace (1953). It has been shown that an inversion's role in a coadapted system cannot be predicted from cytological evidence alone without confirmatory crossover data. This was demonstrated for the Standard/Pikes Peak heterozygote by the presence of only one double crossover in the total of 6105 flies. The relationship of this finding to the formation of stabilized coadapted gene combinations between the ST and PP arrangements is discussed. Since Pikes Peak is a large simple inversion with respect to Standard, two strand double crossovers are expected. This almost complete lack of crossing over cannot be explained on the basis of any simple mechanism of crossover suppression. In addition, data are presented for the crossing over between ST and a newly discovered simple, third chromosome inversion.

ACKNOWLEDGEMENTS

The author is indebted to Professor Carl Epling for providing the VF strain and to Professor Th. Dobzhansky for providing the strain of PP.

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Vol. XC, No. 850 The American Naturalist January-February, 1956

FLORAL ISOLATION IN PENSTEMON[1]

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In cismontane southern California there occur four species of the section Peltanthera of Penstemon (Scrophulariaceae). These four species are conspicuous in themselves and are all conspicuously different from one another, yet they are so closely related that they are apparently interfertile in all possible directions. While each species is different in its habitat preferences, three of the six possible ecologically sympatric combinations are of common occurence, two may be found fairly frequently, and only one has not been found at all. Despite the relative laxity of the genetic and ecological barriers between these species, there is little tendency to hybridize and no tendency to become swamped evident in any of them. Each of these four species is a geographic representative of a distinct species-group with similar morphological and adaptive characteristics, other members of which meet in other areas over the range of the section to which they belong with the same lack of important hybridization. It is apparent that some additional isolating mechanism is contributing greatly to the prevention of genetic amalgamation of these species; the purpose of the present paper is to inquire into the nature of that isolation.

THE FOUR SPECIES OF PENSTEMON

Penstemon centranthifolius is characterized by bright scarlet flowers about an inch long with narrowly tubular shapes and short, erect, sub-equal limbs. The flowers are borne on lax pedicels in a strict thyrsoid or racemose inflorescence that may occupy half or more of the length of the erect, unbranched stem, and they contrast strongly with the glaucous foliage. This species is ecologically tolerant and widespread in southern and west-central California and northern Baja California.

The other extreme of floral morphology is represented by *Penstemon grinnellii,* whose flower has a short, narrow tube but a broadly and abruptly expanded throat and ample lips. The flower is white suffused with pink or blue in the upper throat and, particularly, on the lower lip, and it has a conspicuously exserted staminode which is strongly bearded. The plant is low, branched, and spreading, the herbage is dark green, and the broadly thyrsoid inflorescence is often rather leafy. This species is an inhabitant

[1]The following paper represents a portion of a doctoral thesis prepared at the Claremont Graduate School, Claremont, California. The essentials of this paper were presented at the Annual Meeting of the Society for the Study of Evolution in Austin, Texas, on April 8, 1955, under the same title.

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of open sites in the montane yellow pine forest, which it frequently shares with *P. centranthifolius;* to the north of the area under consideration it is also found at lower elevations in other communities.

The other two species are intermediate in the shapes of their flowers. *Penstemon spectabilis* has a longer tube than *P. grinnellii,* but its throat is only moderately expanded, its staminode is glabrous, and its flowers are various shades of bright blue or purple. This species is a tall, erect, branched perennial with dark green foliage and an ample thyrsoid inflorescence. It is a pioneer in disturbed sites in the chaparral and coastal sage scrub communities, where it is often accompanied by *P. centranthifolius,* and where it may occasionally meet *P. grinnellii* also.

The fourth species in cismontane southern California is *Penstemon clevelandii,* whose flowers have essentially the same shape as those of *P. spectabilis,* but are significantly smaller and of intense magenta colors. Its staminode is usually short-bearded, its habit is low and spreading, and its inflorescence is moderately ample. This species is typically found in the desert-edge canyons in chaparral and pinyon-juniper woods, where it frequently meets *P. centranthifolius,* occasionally meets *P. spectabilis,* but rarely if ever comes into contact with *P. grinnellii.*

POLLINATING AGENTS

With such diverse flower types, ranging from narrowly tubular to broadly open throats, and such different colors, from scarlet through magenta and blue-purple to nearly white, it seems logical to expect diverse pollinating agents. This expectation is confirmed by field observations.

The large-flowered *Penstemon grinnellii* is well adapted for normal pollination by large carpenter bees of the genus Xylocopa (Apidae) (Merritt 1897, Grant unpubl., Straw 1955a), of which two species (*XX. californica* and *orpifex*) are regularly found attending these flowers. Their stout bodies nearly fill the cavity of the throat of this Penstemon as they feed on nectar hidden in the tube. The bees' short probosces are matched by the short tube length of the flower, and angled grooves along the corners of the lower lips provide strategically placed footrests for the bees also. In entering the throat, pollen is abundantly dusted onto the thick hairs of the upper thorax and first segments of the abdomen of the bee, and transfer to the receptive stigmas in the second stage of development of the protandrous flower is quite inevitable. Because of their size, the carpenter bees are unable to enter the corollas of any of the other three species mentioned here, and in the cismontane region are thus quite exclusively the pollinators of this one Penstemon taxon.

Penstemon spectabilis, the next-largest-flowered species, is visited abundantly throughout its range by wasps of the genus Pseudomasaris (Vespidae). Two species have been collected, of which *Ps. vespoides* is rather common, *Ps. wheeleri* rare. These wasps are unusual in that they provision their nests with pollen rather than animal materials (Hicks 1927). Although they range widely over the western United States, the larger

species of this wasp genus appear to be restricted to Penstemon flowers of the size, shape, and blue colors of *P. spectabilis,* and they have been recorded at a number of these other taxa in other areas (Cooper and Bequaert 1950, Cooper 1952, Straw unpubl.). Although they are physically able to enter *Penstemon grinnellii* and feed on its nectar, *Pseudomasaris* species have never been observed to do so; from the other two Penstemons with which we are here concerned they are limited by their size. Thus, the normal pollinator of this Penstemon seems also to be restricted to it within its range.

There is relatively little information available concerning the pollination of *Penstemon clevelandii* on a quantitative basis, but it is visited and pollinated by both solitary bees (Anthophoridae) and hummingbirds.

Each of the aforementioned three insect-pollinated species is visited by a variety of other bees as well, especially small leafcutters (Megachilidae), solitary bees, and bumblebees. For various reasons (size, habits, etc.) which there is not room to discuss in detail here, most of these other bees are considered relatively (but not totally) ineffective pollinators.

The fourth species, *Penstemon centranthifolius,* is a "typical" hummingbird flower, and is visited by both Anna and Costa hummingbirds. The regular outline of the limb surrounding the orifice furnishes a target for the bill, and the narrow tube serves as a guide toward the nectar, as well as to direct the bill past the anthers and stigma to effect pollination. The very short limb and small diameter of the throat in this species prevent most insects from entering, although some of the very small leafcutters, e.g., *Hoplitis producta,* sometimes enter the corolla and take pollen. On the whole, however, insect visits to *P. centranthifolius* have been observed only infrequently.

Although it seems apparent that each of these four species of Penstemon has its own normal pollinators, carpenter bees, wasps, solitary bees, and hummingbirds, there still remains the crucial problem as to how exclusive these pollinators are. In nature the most common species-pairs found together include *P. centranthifolius* as one of their members; that is to say, a hummingbird-pollinated and an insect-pollinated species are found together more frequently than are two insect-pollinated species. Because of the narrow shape and small orifice diameter of *P. centranthifolius* few insect visitors to the other species are able to enter it, and of those that are able, few have been observed to do so. It has been determined from spectrophotometric analysis of the color of *P. centranthifolius* that almost the entire reflectance spectrum of this corolla lies in the red bands (Straw 1955a), beyond the limits of visibility of such bees as have been studied in this regard (von Frisch 1914, Bertholf 1931, *et. al.*). Assuming an essential analogy to human vision (Griffin 1953), this means that to the bee this corolla must be nearly without color, that is, black or some very dull shade. Such colors do not seem regularly to attract the day-active bees. Insect pollination of this scarlet-flowered species thus is strongly inhibited.

Of the insect-pollinated species that might be found together, the normal carpenter bee pollinators of *P. grinnellii* and the masarid wasp pollinators of *P. spectabilis* are limited to their own preferred species by their larger sizes and, in the case of the wasps, by their essential flower constancy. Although *P. grinnellii* grows well within the altitudinal range of *Pseudomasaris,* the wasp has never been observed at that species. The preference of the wasp for corollas of the blue color and moderate size exemplified by *P. spectabilis* seems to be exclusive.

Concerning the bees that visit *Penstemon clevelandii* we have little information beyond the fact that none of the species found is restricted to this flower, but may visit frequently the flowers of other species of Penstemon and other genera as well. This is particularly true of the many megachilid bees, such as species of Osmia and Anthocopa, that are widely distributed throughout this region. We cannot conclude from this that these bees are individually promiscuous, however. On the contrary, they may be very constant to a single kind of flower over any given period of time, just as the honeybee and many bumblebees are known to be (Grant 1950). Michener (1953) has presented evidence that one species of leafcutter (*Megachile brevis*) is quite constant to a single species of flower while collecting pollen, but may be promiscuous when feeding on nectar only. At least one leafcutter, *Ashmeadiella australis,* is restricted to Penstemon (Michener 1939), and in southern California, apparently to *P. grinnellii;* its habits are apparently very constant.

This leaves for consideration the hummingbirds, of which it is well known that, although they may be the almost exclusive pollinators of *P. centranthifolius,* they are by no means restricted either to this species or to this genus of plants. Close observations of the birds in mixed colonies of Penstemon has lead to the belief that the hummingbirds are individually relatively constant to single feeding locales and, over the minimum period of a single feeding flight, to a single species of flower. This conclusion is not out of line with other detailed studies of the habits of hummingbirds (e.g., Bené 1942, Wagner 1946), despite the birds' reputations for promiscuity. Hummingbirds have been seen to cross from one Penstemon species to another on consecutive feeding flights, however, and by such acts might cause hybridizations. Several factors may tend to minimize the chances of illegitimate pollinations under these circumstances. On the one hand, the birds are seen frequently to clean their bills during the pauses between flights, which, if it does not eliminate, must at least greatly reduce the amount of pollen that may be carried from one species to another over the intervening period. On the other hand, while it is almost impossible for a hummingbird to probe a flower of *P. centranthifolius* in the proper stage without obtaining a considerable amount of pollen on its bill or placing pollen on the stigma, in the larger, insect-pollinated species the bill may touch the essential organs only accidentally. In *P. grinnellii* the birds have been observed habitually to enter the corollas from so oblique an angle that pollen might be placed on the stigma only with extreme rarity.

ISOLATING MECHANISMS

It seems evident that this whole complex of ethological and mechanical characteristics of the flowers and their pollinators has helped to keep the four species of Penstemon mentioned here quite discrete from one another, and has permitted the sympatric occurrence of species between which genetic, ecological, temporal, and other barriers are quite inefficient. Since the mechanism depends on the habits and discriminatory abilities of the animal pollinators as well as on a number of purely mechanical factors, it is thought best to refer to this system as "floral isolation" (Grant 1949), in a rank coordinate with the other types of isolating mechanisms generally distinguished in biological systems (e.g., Stebbins 1950, Chapter 6; Riley 1952).

In some situations floral isolation may be nearly 100 per cent effective, as, apparently, in a case described in Delphinium by Epling (1947, as discussed by Grant 1949: 90 ff.). Complete dependence upon one specific pollinator may, however, result in the infertility of a colony if that normal pollinator should for some reason fail to appear, and in the long run might lead to the extinction of the species as a result of overspecialization. In these species of Penstemon floral isolation is not so highly developed as to run this risk, since insects other than their normal pollinators may, with a frequency dependent largely upon their size, sometimes pollinate them. These species are also all perennials, to which a reduced seed set in any one year would not be so destructive an event as if they were annuals.

Successful hybridization, furthermore, is not unknown. One hybrid, *Penstemon centranthifolius* × *spectabilis* (= *P.* × *parishii*), is rather common in the areas where its parents occur together, and is a distinctive and easily recognizable entity. A second hybrid, *P. centranthifolius* × *grinnellii* (= *P.* × *dubius*), has been found twice. The appearances of such characters as bearded staminodes in colonies of *PP. spectabilis* and *centranthifolius* and modifications in the corolla proportions in *PP. centranthifolius* and *clevelandii* seem to indicate further gene flow between other species. The characters of the plants in these last cases are so confused that it is at present impossible confidently to defend the hypothesis of introgression which is, nevertheless, tentatively advanced.

We must not interpret this evidence of gene exchange as detrimental to the hypothesis of floral isolation, however. In the species where no clear-cut cases of hybridization are known, the evidences for introgression are of a questionable nature also, while of the two natural hybrids that are known, one has been found only twice in an area where its parents overlap that has been carefully explored by botanists for many years, and the other is deserving of special consideration.

The case of the rather common occurrence of *Penstemon centranthifolius* × *spectabilis* is particularly interesting. About ten hybrid individuals have been seen in the field during the past two years, and in every case they were in very small mixed colonies of the parents (usually fewer than thirty individuals of both kinds) rather than in the more extensive mixed colonies that may also be found. It is suggested that the small numbers of plants

of any one species available for feeding upon in these small aggregations may cause the normally constant pollinators, here probably hummingbirds, to break their constancy and seek food in the flowers of the other species, thereby making illegitimate pollinations. Colony size appears to affect the constancy of insect pollinators in a similar manner.

The colonies in which hybrids are found are somewhat unusual also, in that in no case seen by me has it been possible to identify in the field or to detect by special means what might be called a hybrid swarm. The colonies observed have regularly contained the two parents and one or two obvious hybrids, but none of the intergrading segregates or backcrosses one would expect to find. If either segregates or backcrosses have been present their morphology has been such that they could not reliably be distinguished from the parents or the hybrids, and the plants all fall easily into the three classes. The hybrid is highly fertile (average of ten plants, 65.7 per cent good pollen, as measured by stainability in aniline blue and lacto-phenol) and produces some viable seed. Floral isolation, however, discriminates strongly against these individuals, and in the field they are relatively little visited by the insects and hummingbirds that are attracted to their parents in great numbers. Where the hybrids are visited and backcrossed, this stringent selection appears quickly to draw the hybrid types into conformity with one of the parents. This situation is thus considered to be another example of the mechanism described by Grant (1952) in Aquilegia for the selection toward the parental types in a hybrid swarm. Its only evident result seems to be a rather large variability pattern in *P. spectabilis*. A further mechanism, for the production of new species through hybridization and selective pollination, is described elsewhere (Straw 1955b).

SUMMARY

Four species of Penstemon section Peltanthera in cismontane southern California are very different in appearance and occur in several sympatric combinations. Despite the fact that they are largely interfertile, hybridization is relatively rare. Field studies have shown that each of the species has its own normal range of pollinators, and it is concluded that floral isolation adequately restricts the hybridization that might lead to swamping or merging of the species.

ACKNOWLEGDEMENTS

The author is pleased to recognize the valuable assistance and criticisms of Dr. Verne Grant both through the long course of his research and in the preparation of this paper. He is indebted also to Drs. Th. Dobzhansky and Alden Miller for specific criticisms concerning the paper. The research basic to this paper has been supported for the past two years through Fellowships from the National Science Foundation, whose aid is gratefully acknowledged.

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Vol. XC, No. 850 The American Naturalist January–February, 1956

"NATURAL IMPLANTATION" OF A LETHAL MUTATION IN *DROSOPHILA MELANOGASTER*

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Many lethal mutations are characterized by different reactions of the various organ systems. Certain parts may become entirely abnormal, whereas others are only slightly affected. The great variation in developmental potencies of the separate parts can be studied by isolating them from their surroundings in the lethal individual. This has been done for a number of lethal genotypes, mainly by Hadorn and his collaborators (for detailed references see Hadorn 1950, 1951). Observations were made on the capacity for autonomous differentiation of primordia from lethal larvae when transplanted into genetically normal hosts. Thereby two major categories of effects can be distinguished. Certain organ systems do not develop beyond the stage they would have reached in the lethal individual; cell factors that are intrinsic or at least determined prior to transplantation thus seem responsible for the inhibited capacity of differentiation in these cases. Other structures, however, seem to develop normally and can sometimes even be shown to function normally.

This elegant technique has produced some very interesting information, but its application offers certain restrictions. Transplantation is only feasible in late larval stages and hence mutations which exert their killing effects earlier in development cannot be studied by this method. It is also practically impossible to study whole organ-systems for this would involve gross transplantations which could hardly be made because of their complexity. Furthermore, as Hadorn (1951) points out, failure of development after transplantation may lead us to the erroneous conclusion of direct damage by some intrinsic factors. Exchange of transplants is restricted to the larval stages only, and therefore, transference to normal surroundings may have come too late to undo irreparable damage to certain systems, which had already been exposed for too long a time to the unfavorable extrinsic influences of the lethal milieu.

More information as to the latter problem has been obtained by a very different approach. Demerec (1934), Ephrussi (1934), and Stern (1935) used stocks which increased the frequency of somatic segregation, crossing-over,

*This work was supported in part by a research grant for work of H. J. Muller and associates from the U. S. Atomic Energy Commission (AT(11-1)-195), and a grant from the British Empire Cancer Campaign; present address: Department of Zoology, Indiana University, Bloomington, Indiana, U. S. A.

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‡Contribution No. 586.

elimination or non-disjunction, in order to obtain small patches of tissue homo- or hemizygous for both visible mutations and recessive lethal deficiencies. In these cases the lethal-bearing cells were thus surrounded by normal tissue from the onset of development. Under such conditions some lethals were viable, while others caused inviability even in single cells that were embedded in a normal blastema. Therefore one may conclude that certain lethals are characterized by a cell-intrinsic cell-lethality.

Another possibility for studying the effect of lethal gene action would be by an analysis of sex-linked recessive lethals in gynanders. This method relies on the fact that an egg one of whose X chromosomes gets lost or undergoes a gross deletion during an early cleavage stage gives rise to a gynander or mosaic individual. If the maternal X-chromosome in addition to visible markers carries a recessive lethal and the paternal X-chromosome is lost during the first cleavage it would be possible, theoretically, to obtain flies half of whose bodies are hemizygous for the lethal in question. Loss of the paternal X during a subsequent division would result in smaller hemizygous areas. Supposing now, that this lethal is not a cell-lethal, the problem arises, how large an area of hemizygous lethal-bearing tissues could be borne with impunity. The use of gynanders as a means for studying sexual differentiation in *Drosophila* was originally suggested by Muller (personal communication). Sturtevant (1920) has used gynanders to show that the eye color mutation, vermilion, undergoes non-autonomous differentiation, unlike most of the other eye color mutants in the fruit fly. Other workers (Patterson and Stone 1938, Stern and Hannah 1950, Bonnier 1950, Novitski 1951, Hannah 1953, and Stern 1954) have also utilized this technique to determine the degree of autonomy exhibited during the growth, differentiation, and functioning of various other imaginal tissues in *Drosophila*.

In this study we used the mutation lethal(1) melanoma-like (*l*ml,1-1.0) (Oster, 1952). Males carrying *l*ml die in the third larval instar with many black melanotic inclusions uniformly distributed throughout the body. We thereby hoped to obtain some information on the origin and distribution of these melanotic "tumors" in the mosaic individuals. Similar studies on a number of other sex-linked lethals are in progress now.

MATERIALS AND METHODS

Previously Muller (1940, 1954) had shown that irradiation increases the frequency of ring-X-chromosome loss, such as the one designated X^{c2}, and the subsequent formation of gynanders. For this reason males containing X^{c2} were irradiated in two series of experiments with 4000 to 5000 r and mated to females of the composition: $\frac{\text{y } l\text{ml w In49 f. } Y^{s}}{\text{sc}^{s1}\text{ B InS}}$. In the second series the females were aged for ten or more days, as Brown and Hannah (1952) had shown that the ring-X-chromosome is eliminated with a higher frequency when introduced into eggs derived from aged females. After three

days the male parents were discarded and the females were transferred to fresh culture bottles in which they were allowed to lay eggs for an additional five days.

Three F_1 classes of flies were obtained:

$\frac{y\ l\text{ml w In49 f. } Y^s}{X^{c2}}$	$\frac{sc^{Sl}\ B\ InS}{sc\ X^{c2}}$	$\frac{sc^{Sl}\ B\ InS}{Y}$
normal (+) ♀	Bar (B) ♀	scute Bar (sc B) ♂

The fourth class of flies, that is, males of the composition y *l*ml w In49 f.Y^s, did not survive because of the presence of *l*ml.

Yellow (y), white (w), and forked (f) were used as visible markers for the detection of hemizygous areas in the normal class of F_1 females. In the Bar (B) class of females, scute (sc) and Bar (B), served the same purpose. The presence of inversions (designated sc^{Sl} and InS) served to suppress somatic crossing-over in the F_1 offspring.

RESULTS

The results are summarized in the accompanying tables.

TABLE 1

The frequency of gynanders and mosaics in the offspring from the cross:

$$P_1\ \frac{y\ l\text{ml w In49 f. } Y^s}{sc^{Sl}\ B\ InS}\ ♀ \times \frac{X^{c2}}{\longleftarrow}\ ♂$$

F_1

	+♀	B♀	sc B♂	Gynanders and/or Mosaic Individuals	
				+♀	B♀
Series I	1867	1731	724	No. 1: ½ of abdomen yellow; external genitalia male-like; small melanotic area near genitalia.	No. 1: 1 eye hemizygous Bar; whole head scute.
					No. 2: 1 eye hemizygous Bar; whole head scute; 1 sex comb.
					No. 3: ½ of thorax scute; 1 wing shorter (i.e., male-like).
					No. 4: ½ of thorax scute; 1 wing shorter (i.e., male-like).
				$\frac{1}{1868} = 0.053\%$	$\frac{4}{1735} = 0.23\%$

TABLE 2

				Gynanders and/or Mosaic Individuals	
	+♀	B♀	sc B♂	+♀	B♀
Series II	1129	953	1122	No. 1: less than 1/10 of 1 eye white.	No. 1: external genitalia male-like.
				No. 2: 1/10 of 1 eye white.	No. 2: orbital, ocellar, vertical, post-vertical and scutellar bristles missing.
				No. 3: 1/5 of 1 eye white.	No. 3: 1/2 of thorax scute.
				No. 4: 1/5 of 1 eye white.	No. 4: 1/2 of scutellum scute; 1 wing shorter (i.e., male-like).
				No. 5: 2/5 of 1 eye white.	No. 5: sternite bristles on 1/2 of abdomen reduced in size; 1 wing shorter (i.e., male-like).
				No. 6: 7/8 of 1 eye white.	No. 6: 1/2 of abdominal tergite pigmentation male-like; 1 wing shorter (i.e., male-like).
				No. 7: 1 eye all white with the exception of a few red facets; 1/2 of head yellow and forked.	No. 7: 1 eye hemizygous Bar; 1/2 of head scute.
					No. 8: 1 eye hemizygous Bar; thoracic bristles strongly reduced in number; sternite bristles reduced in size and number; 1 sex comb; 1 wing shorter (i.e., male-like).
				7/1136 = 0.62%	8/961 = 0.83%

CONCLUSIONS AND DISCUSSION

The observation of various mosaic flies among the "normal" (y *l*ml w In49 f.Y^{s}/X^{c2}) class of females shows that individuals with patches presumably hemizygous for *l*ml can survive to the imaginal stage. Thus, the mutant, *l*ml, at least in the hypodermal cells, does not act as a "cell-lethal." Gynanders with sizeable areas of hemizygous tissue were, however, completely missing among the "normal" females. A comparison of the size of the mosaic parts in the two classes of females shows that the hemizygous areas were much smaller in the "normal" than in the Bar-eyed class of females. It seems as if in individuals with sizeable areas hemizygous for *l*ml, the deleterious effects of this gene prevent the developing animal from

reaching maturity. Another way of explaining the limited size of patches hemizygous for *l*ml might be to assume that there is a retarded growth in mutant tissues, as compared with the growth rate of normal cells. Observations on the developmental rate of ordinary *l*ml males, however, do not support this hypothesis. As the size of the mosaic parts can be correlated with the time of elimination of the ring-chromosome it is clear that *l*ml exerts its killing effects mainly in those cases where the paternal X-chromosome is lost at a relatively early cleavage. On the other hand, those individuals which have lost the ring during a late cleavage (that is, those having small hemizygous areas) are capable of surviving to adulthood.

Considering the mosaics in the class of normal females in Series II it is apparent that in the vast majority of these cases only the marker white (*w*) shows up. Some of these mosaics may possibly be due to deficiencies for the white locus, caused by the heavy irradiation of the paternal X-chromosome. They therefore may still contain the normal allele of *l*ml. But the observation of a mosaic fly showing all markers, namely yellow, white, and forked (no. 7 in Series II), and the fact that *l*ml is closely linked to white, both support the conclusion that lethal(1) melanoma-like does not act as a cell-lethal.

The unusually high frequency of individuals with small mosaic areas warrants a further consideration in view of the previous findings of Battacharya (working under Muller's direction), who failed to detect any increase in the number of small patches of white facets in the eyes of the female offspring of irradiated ring-X males (Muller 1940, 1954, Battacharya, 1950). In the first series, our results were essentially similar, that is, the mosaic individuals which we found contained large hemizygous areas. However, in our second series, in which we had used aged females, we found many cases of individuals bearing small mosaic areas both among the normal and Bar-eyed female offspring. In all probability they were caused by the aging of the females, mediated perhaps by some unknown cytoplasmic factor(s), and not due to the radiation. This is in line with the results of Brown and Hannah (1952), who reported a similar increase in the number of small mosaic areas in the offspring of crosses of non-irradiated ring-X males mated to aged females.

This incidental finding which is under further study can now be made use of in studies similar to the one described above for determining whether a particular mutant is a cell-lethal in the same cross as that used for studying the mutant's behavior in gynanders. That is, irradiation of the ring-X would result in gynanders if flies bearing sizeable areas of the particular lethal being studied are viable, while the appearance of small mosaic areas produced by the aging of the female parent would indicate that the lethal in question is not a cell-lethal.

SUMMARY

A method is described for the production of mosaic flies with tissues hemizygous for sex-linked recessive lethals. Our investigation deals with

the mutant, lethal(1) melanoma-like (*l*ml, 1-1.0). Individuals with patches hemizygous for this sex-linked lethal are viable, but it was observed that these areas were much smaller than mosaic parts in gynanders of the control class. It therefore appears that *l*ml does not act as a cell-lethal but that its deleterious effects prevent the animal from reaching the imaginal stage if sizeable areas are under the influence of this gene.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Professor H. J. Muller and Dr. C. Auerbach for many stimulating discussions during the course of the work and to Professor C. H. Waddington for kindly providing the facilities with which the latter part of this study was carried out.

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LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

COMMENTS ON DR. WHITE'S ARTICLE ON TISSUE CULTURE

Dear Sir,

I have read with much interest Dr. Philip White's stimulating article on "Cellular Biology and Tissue Culture."

Dr. White states that Tissue Culture has not entirely fulfilled the extravagantly high hopes with which it was originally regarded, and I think that there are two main reasons for this. In some ways the method was born before its time. When it was found that living cells could be cultivated outside the body, it seemed at first that we had been provided with perfect material for studying the physiology of growth. In practice, however, it proved extremely difficult to obtain accurate quantitative information about the metabolism of tissue cultures, because the microchemical techniques required for such delicate work have only become available during the past few years. To some extent this technical limitation also applied to the study of individual living cells in vitro, but the recent development of ultra-violet and interference microscopy, for which the thin, well spread cells of tissue cultures are ideally suited, has opened a new approach to the quantitative investigation of many aspects of cellular physiology.

In the second place, for many years there was an unfortunate tendency to elevate Tissue Culture to the status of a separate branch of biology, so that its true function as merely "a versatile tool" (to quote Dr. White) was not always appreciated. I myself have often been approached by people who wished to learn Tissue Culture without having any definite problem in view and who were obviously quite unaware of either the possibilities or the limitations of the technique. In some laboratories this woolly attitude of mind has meant that Tissue Culture has been asked the wrong questions or, indeed, no questions at all; a mass of valueless observations have resulted, while many important problems which the technique might have solved have been neglected.

As Dr. White points out, in the past certain tissue culturists "permitted themselves to be somewhat more fascinated by the techniques than by their proper objectives." The perfecting of a Tissue Culture method is a will-o'-the-wisp which can be pursued indefinitely with much personal enjoyment but little scientific advantage, and in general it is more profitable to develop the simplest possible technique that will serve the investigator's purpose and then concentrate on the research itself. At the present time there tends to be a cleavage between the 'simple' and the 'complex' schools

of Tissue Culture, but in fact the complexity of the procedure should depend entirely on the nature of the problem to be studied. On the one hand we should not, out of technical snobbery, reject a method which would be entirely suitable for a certain investigation, just because it happens to be simple and old fashioned, but on the other hand we should recognise the fact that some types of research may demand an elaborate and costly organisation. The important thing is not to waste time and money on a complicated technique where a simple one will do equally well (and sometimes much better).

During the past decade, Tissue Culture has been contributing to biological and medical research with ever increasing success. The technique is being used in a much more realistic and fruitful way than formerly, and as Dr. White has indicated, it is now being applied to many widely different problems which cannot be satisfactorily studied in the intact organism. This renaissance dates from the Hershey Conference in 1946, and owes much to the imaginative and enterprising officers of the American Tissue Culture Association.

Yours etc.,

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Sept. 1, 1955

CROSSING OVER WITHIN INVERTED REGIONS IN CHIRONOMUS.

In those animals in which inversion heterozygosity is common, and in which there is crossing over in the male, it has been supposed that chiasmata must nearly always be confined to those regions of the chromosome in which there are no inversions (Philip, 1942; White, 1954). Though the abnormal chromatids which result from crossing over in inversion-loops are known in some cases to be eliminated in the female (Sturtevant and Beadle, 1936; Carson, 1946), the same mechanism cannot exist in the male.

Chiasmata can be seen in the testes of *Chironomus thummi*, *C. dorsalis*, *C. annularius* and *C. tentans*. Crossing over is known definitely to take place in both sexes in *C. tentans* (Beermann, 1952), and it follows that there may be crossing over between certain inverted sequences which would lead to the production of abnormal chromosomes. In the left arm of chromosome 2 in this species (Beermann, 1955) there are several different, mutually exclusive, sequences. Crossing over in individuals heterozygous for two of these sequences will lead to the formation of a chromosome, not dicentric, with a short duplication and deficiency. These, presumably, cannot be eliminated in oogenesis as there will be no anaphase bridge, and most of the abnormal chromosomes must eventually be eliminated by selection. Nevertheless, I have found one larva among 1600 carrying such a chromosome. It is apparent then that crossing over does occur between some arrangements, and that at least occasionally abnormal chromosomes are produced which are not lethal.

No evidence of crossing over within the inverted region of an inversion heterozygote has been reported in male Chironomidae, though a study of spermatogenesis in *C. dorsalis* has been made in which no evidence of bridges and fragments was seen (Philip, 1942). I have examined larvae of the same species from a population in which the frequency of one of the inversion heterozygotes approached 50%. A number of suitable squash-preparations was made of the testes of early prepupae. One of these preparations was of the testis of a prepupa heterozygous (judged from its giant chromosomes) for three inversions, one in each of the two longest chromosomes (1 and 2) and one in the shortest (4). Seven good examples of bridges and fragments were seen; the fragments were of two types, one long (Fig. 1) and the other short (Fig. 2). Chromosome 4 is easily identified, so that the fragments must have been the result of crossing over within each of the remaining inversions. In a second preparation in which there was only one of these inversions (chromosome 1), the fragments were short, so that the longer fragment in the first preparation must correspond to the inversion in chromosome 2. No quantitative data on the frequency with which bridges and fragments are formed are yet available, and may be difficult to get as their presence, or absence, in a given preparation may depend on the age of the testis.

In all populations of *C. dorsalis* so far studied inversion heterozygotes have been found at appreciable frequencies, and consequently the deleteri-

FIG. 1 FIG. 2

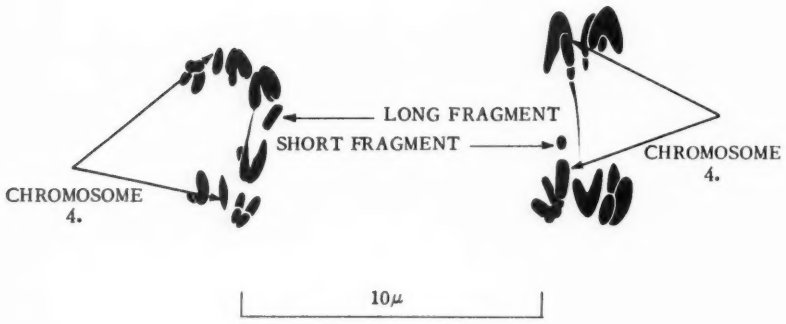
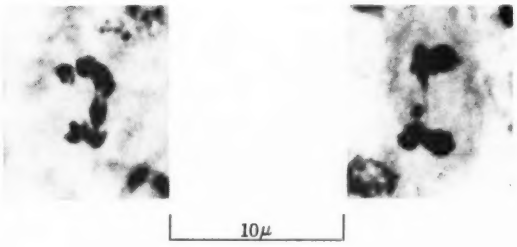


FIG. 1 FIG. 2



CHIRONOMUS DORSALIS—BRIDGES AND FRAGMENTS IN TESTIS OF LARVA HETEROZYGOUS FOR INVERSIONS IN THREE CHROMOSOMES

ous effects of crossing over for the population cannot be mitigated by the fact that inversions are rare. Furthermore, the number of heterozygotes in which crossing over will produce abnormal chromosomes without the formation of a bridge at meiotic anaphase is also quite high in some populations. Unless crossing over in inversion heterozygotes is very rare the selective values of the various combinations of chromosomes arrangements must be high enough to outweigh this disadvantage. Further details will be published elsewhere.

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Sept. 4, 1955

THE
AMERICAN NATURALIST

Vol. XC No. 850

Supplement to January-February, 1955
Published for

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Oak Ridge National Laboratory,
Oak Ridge, Tennessee

COMPARISON OF X-RAY-INDUCED MUTATION RATES IN DROSOPHILA AND MICE

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Vol. XC, No. 850 The American Naturalist January-February, 1956

COMPARISON OF X-RAY-INDUCED MUTATION RATES IN DROSOPHILA AND MICE[1]

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Until recent years, estimates of genetic hazards of ionizing radiation in man were based primarily on information obtained from Drosophila. Our investigations (Russell, 1951) on radiation-induced mutation rates in mice, and a comparison of the results with as nearly similar data as were available for Drosophila, showed a higher mean rate in the mouse, and led to the conclusion "that estimates of human hazards based on Drosophila mutation rates may be too low." It was recognized that the Drosophila data that were available were not ideally suited for comparison with the mouse data. Thus, the mouse mutation rates were determined for irradiated spermatogonia, utilizing seven specific autosomal loci, and there was no information on specific-loci rates for irradiated spermatogonia in Drosophila, although there was information on specific-loci rates for irradiated spermatozoa. We considered this a serious lack and we, therefore, suggested and supported the study made in our laboratory by Alexander (1954) which was designed specifically to provide Drosophila data more suitable for comparison with the mouse. In this investigation, the mutation rates were determined: (a) at specific loci, (b) on autosomes, (c) for irradiated spermatogonia as well as spermatozoa, (d) with essentially the same genetic technique and method of scoring the mutations as had been used on the mice, and (e) with the same X-ray machine and method of dosimetry as that used in the mouse experiment. Alexander mentions the mouse mutation rate in her publication, but she does not make a detailed comparison of her data with the mouse data, it having been decided to postpone this until additional mouse data were published. It now appears important to make a detailed comparison without further delay. One reason for this is the suddenly increased interest in genetic hazards of radiation. Another reason is the recent paper by Ives (1954) in which he discusses radiation-induced mutation rates in Drosophila and mice, using our mouse data, and Drosophila data of Alexander (1954)[2] and himself. He concludes "that for the present the radiation-induced mutation rate per r per locus appears to be similar in flies and mice." Thus Ives disagrees with the conclusion I stated in 1951, that comparison of the mouse data with similar data from Drosophila shows a higher induced mutation rate in the mouse. Since I believe the new Drosophila data of both Alexander and Ives

[1]Work performed under Contract No. W-7405-Eng-26 for the U. S. Atomic Energy Commission.

[2]Ives' paper does not include a reference to this particular article, which perhaps had not appeared when Ives submitted his paper for publication. The only publication of Alexander's that he cites is an earlier abstract. However, it is clear from Ives' treatment of Alexander's data that he had access to a much more extended report of her work than was given in her abstract.

support my original contention, and since this question has a bearing on the important problem of estimating genetic hazards of radiation in man, it seems necessary to discuss the species comparison at some length and point out what I consider to be major errors in Ives' argument.

Perhaps the most serious fault of Ives' paper is that nowhere does it contain a discussion of Alexander's spermatogonia data. In order to ensure that these data came from irradiated spermatogonia, Alexander treated the flies in early larval stage. Although this involved painstaking work, it was undertaken because, as has already been pointed out, it was considered important to have Drosophila data comparable to the mouse data which, because of the importance of the spermatogonial stage in human hazards, had been obtained from irradiated spermatogonia. Yet Ives, despite the fact that his paper is devoted to a consideration of the species comparison, completely ignores the Drosophila spermatogonia results and discusses only the data on spermatozoa which Alexander obtained for comparison with her spermatogonia results and for future comparison with mouse spermatozoa results when these become extensive enough. Throughout his paper Ives uses only the Drosophila spermatozoa mutation rates for comparison with the mouse rate in spermatogonia, which, incidentally, he wrongly calls "immature sperm." Since he mentions that I pointed out the complication in making such a comparison, it is clear that he should have been aware of the desirability of having spermatogonia data in Drosophila. It is, therefore, especially hard to understand why he does not discuss Alexander's spermatogonia rate and why he even fails to mention that she worked on spermatogonia as well as on spermatozoa. Even if nothing were known about the quantitative and qualitative difference between mutants recovered from spermatozoa and spermatogonia, it would seem to be good judgment to use the same cell stage in making comparisons between species. Actually, since the work of Hertwig (reviewed by Russell, 1954) much has been known about the difference in results obtained from irradiated spermatozoa and spermatogonia in the mouse. Thus, there is a virtual absence of dominant lethals and translocations in offspring obtained from gametes that were irradiated as spermatogonia with doses that cause a high incidence of these aberrations in irradiated spermatozoa. There are, therefore, actual experimental data showing that, whenever possible, it is, indeed, desirable to use the same cell stage in making comparisons.

Ives' failure to appreciate the qualitative difference in results obtained from irradiated spermatozoa and irradiated spermatogonia leads him into making comparisons that are not valid. For example, he says sterility and inviability of the mutants are "apparently much higher in Drosophila than in mice." Since he is comparing mutants from irradiated spermatozoa in Drosophila with those from irradiated spermatogonia in mice, it cannot be claimed that the difference in behavior of the mutants is a species difference. There is evidence that indicates that a large part, if not all, of the difference may be a result of difference in the cell stage irradiated. For example, it has long been known (see review by Russell, 1954) that sterility, in general, is

common in the offspring resulting from irradiated spermatozoa, but not in the progeny from irradiated spermatogonia. In her paper, Alexander does not comment on the viability and fertility of presumed mutants in her spermatogonia experiment, but it is apparent, from a private communication, that, in these respects, her Drosophila spermatogonia data are much closer to the mouse spermatogonia data than are Drosophila spermatozoa data.

Ives cites a "few cases" in his work where apparent mutations are not inherited. He attributes these to "delayed mutations" which involved only the soma, and, he states that, because of the difference in the stage of development at which separation of the germ line occurs in Drosophila and mice, mutation rates will be differentially biased if these cases are scored only when they are heritable. Regardless of whether Ives' interpretation that these are delayed mutations is correct or not, he again makes the error of comparing Drosophila spermatozoa and mouse spermatogonia. Since no non-heritable cases were found in the entire sample of mutations from irradiated mouse spermatogonia, it can safely be concluded that if there are any heritable induced mutations that are delayed in occurrence until embryonic stages, they must be rare and cannot contribute more than an insignificant amount to the observed mouse mutation rate.

In spite of the difficulty of comparing mutation rate in spermatogonia of one species with that in spermatozoa of another species, it is sometimes necessary to make such comparisons. This was the case in my 1951 report on mouse mutations since, as has already been mentioned, no data were available at that time on specific-loci mutation rates in Drosophila spermatogonia. Since, even now, Drosophila data of this kind are not extensive, it is still of some interest to compare the mouse spermatogonia mutation rate with the Drosophila spermatozoa rate. However, the comparison must be made as it was in my 1951 report, namely, with due regard to the difference between the cell stages. This is not the way in which Ives makes the species comparison. In the first place, as will be discussed later, he makes no allowance for a quantitative difference in mutation rate between the cell stages. In the second place, his attributing the frequent inviability and sterility of mutants from irradiated Drosophila spermatozoa to the peculiarity of the species, rather than to the cell stage irradiated, leads him to make the remarkable specification that the species comparison should be based only on "F_1 results." (His other specification that only autosomal loci be used, had already been met in Alexander's experiment.) What Ives calls "F_1 results" may be defined as the total of all those individuals which, by phenotypic criteria, could be mutants, regardless of whether or not they actually test as such. This seems to me to be entirely the wrong criterion to use for the species comparison. Ives qualifies his criterion to some extent by stating "it is important to attempt the genetic identification of the F_1 mutants in order to determine the accuracy of the observer's classification of the F_1." However, as will be shown, he does not apply this qualification consistently.

The illogicality of Ives' position can be shown in the following manner. If an attempt has been made to test all the presumed mutants genetically, they can then be divided into three main groups: (a) those tested and found to be mutations at the specific loci, (b) those tested and found not to be mutations at the specific loci, i.e., phenocopies and mimics, and (c) those which died before testing or were found to be sterile. It is apparent that a maximum estimate of mutation rate is given by (a) + (c). A better estimate would be obtained if any decision could be made as to what portion of category (c) could be attributed to specific-loci mutations. When this cannot be done, comparison of point mutation rates between Drosophila and mice, or between different sets of Drosophila data, are probably best made on the basis of category (a), rather than on (a) + (c). This will be discussed later. In any case, it would seem clear that category (b) should not be included in estimates of the mutation rate at the specific loci. Yet what Ives is proposing is that category (b) be included and that the mutation rate be estimated from (a) + (b) + (c). It is precisely in this way that he calculates his estimate of the mutation rate from Alexander's data. This is not a small matter. In Alexander's data, category (b) is the largest of the three. Thus, Ives' tabulation of mutations in Alexander's data includes three categories, the largest of which Alexander had proved genetically *not* to be mutations at the specific loci.

What Ives apparently does with his own data is to include all three categories in calculating the mutation rate, but only after some investigation as to the size that (b) would have if no qualifications were applied to taking "F_1 results." As a result of this investigation, he concludes that roughoid-like variants prove to fall in class (b) so frequently that taking only "F_1 results" would lead to major error. Instead of recognizing this as an indication that he should discard the method of taking "F_1 results," he then decides to exclude all of his data on the roughoid locus, although in an earlier paper (Ives, Levine and Yost, 1954) he had not done this. (In the earlier paper, Ives gives what seem clearly to be the same data, but the total number of "res-like mutants" for the 3000-r X-ray dose is listed as 68 instead of the 39 given in the later publication. Thus Ives, himself, demonstrates the unreliability of using "F_1 results.") Peach-type variants also give some trouble, but Ives thinks that "with practice" one can distinguish the true peach-locus mutations by their phenotype. By such means, Ives reduces the size of category (b) in the tabulation of his own "F_1 results." Perhaps he has reduced this category to zero. Since he states that he has tested all the presumed mutants in his work, he should know whether or not it is zero. In any case, if category (b) is not large in his data, it is not surprising that the mutation rate that he obtains from his own results is considerably lower than the spurious rate he calculates from Alexander's data, where he is including all of the large group of variants that were proved not to be mutants. It is in category (b) that the results of different investigators will tend to vary, depending on their personal evaluation of which cases are doubtful enough to require testing. Thus, the difference

between the "F_1 results" of Alexander and Ives indicates not a difference in mutation rates, but simply that Alexander was either more cautious or less practiced than Ives.

The fact that "F_1 results" do not form a reliable basis on which to make comparisons is illustrated by material already published that makes it possible to compare, both on the basis of tested mutations and "F_1 results," different sets of data from the same species, for the same loci and for approximately the same cell stages irradiated. Thus, if Alexander's data are compared with Patterson's (quoted by Alexander) for the seven loci common to both sets of data, the mutation rates based on tested mutations are 5.6×10^{-6} and 3.4×10^{-6}, respectively, while "mutation rates" based on "F_1 results" would be 41.3×10^{-6} and 8.74×10^{-6}, respectively. Similar comparisons between Alexander's and Ives' results cannot be made accurately since it is not possible from the information provided by Ives to determine what proportion of his 39 "mutant F_1" would be comparable to Alexander's tested mutations. However, from the general statement that "in most series half or more of the *res*-type F_1 mutants have failed in retests because of sterility or inviability" one may conclude that a mutation rate based on tested mutations would be something less than half of 14×10^{-6}, and, therefore, presumably in good agreement with Alexander's 6.3×10^{-6} for the same seven loci. A similar comparison based on "F_1 results" instead of tested mutations gives 14.0×10^{-6} for Ives and 24.4×10^{-6} for Alexander. It is apparent that in mutational data that are expected to be in agreement, close agreemant has been found when the results are compared on the basis of tested mutations, but in these same data there can be wide discrepancy in "F_1 results."

Since "F_1 results" are demonstrably unreliable for comparisons made within Drosophila, and on data obtained from the same loci and from irradiation of the same cell stage, it is apparent that, on this account alone, they should not be chosen as the basis for comparisons between different species and between results from irradiation of different cell stages. Furthermore, for comparisons of this sort, "F_1 results" are likely to be even more unreliable. Thus, the proportion of the "F_1 results" falling in category (b), i.e., phenocopies and mimics, is likely to be quite different in an insect and a mammal and in results from irradiation of spermatozoa and spermatogonia. Also the proportion of category (c), the inviable and sterile variants, that is comprised of individuals that do not represent point mutations at the specific loci is likely to be different in results from irradiated spermatozoa and spermatogonia. It may be concluded that when mutation rates for irradiated Drosophila spermatozoa have to be compared with mutation rates for irradiated mouse spermatogonia, the method proposed by Ives, that of using "F_1 results," may lead to serious error.

Considerable space has been devoted to criticizing the above method of comparison because Ives proposes it as the one that should be used in the future. The method that should be used, whenever possible, is to compare the species on the basis of tested mutations. When the comparison is made

between data from irradiated spermatozoa in Drosophila and from irradiated spermatogonia in the mouse, another proviso must be added. This is discussed in the next paragraph.

In my original comparison (Russell, 1951) of specific-loci mutation rates in Drosophila spermatozoa and mouse spermatogonia, allowance was made for the possibility that the Drosophila spermatogonia rate might be lower than the Drosophila spermatozoa rate. On the basis of the data then available, which did not include specific-loci data, the spermatogonia rate was taken as one-half of the spermatozoa rate. Ives objects to this, stating: "I do not feel we know enough about the whole situation yet to justify that procedure." The procedure was justified by the necessity for obtaining as good estimates of human hazards as possible from the existing data. It was recognized that the "situation" had not been adequately explored and, as has already been stated, Alexander's work was started in our laboratory to help remedy the situation. It is hard to see how Ives can justify his own procedure of completely ignoring Alexander's results on this point. Alexander's work provided not only the first data in Drosophila on spermatogonia mutation rates for specific loci, but also data on spermatozoa for the same set of loci. These data show that the spermatogonia rate is, indeed, significantly lower, the point estimate being about one-fourth of the spermatozoa rate. The recent work of Auerbach (1954) indicates a similar magnitude of difference between spermatogonia and spermatozoa rates of mutation to autosomal lethals in Drosophila. Other data of both Auerbach (1954) and Muller (1954) show that certain late stages of spermatogenesis have even higher induced mutation rates than spermatozoa. Therefore, the differential between these stages and spermatogonia is even greater than that between spermatozoa and spermatogonia. Some of the Drosophila data originally regarded as spermatozoa data (and, for convenience, referred to as such in the present paper) may have been comprised partially of data from these more sensitive stages. In any case, all the indications are that the figure of one-half used for the original adjustment of the Drosophila spermatozoa rate for comparison with the mouse spermatogonia rate tended to underestimate the difference between the species rather than, as Ives implies, overestimate it.

Limitations are inherent in any method by which species can be compared. The specific-loci method was chosen for the first phase of the mouse mutation rate studies because it was felt that it would provide the most suitable type of information for species comparisons. However, my 1951 paper did not, by any means, ignore the limitations of the results obtained by this method. In discussing the limitations, Ives states: "It is evident that in both organisms some loci mutate more frequently than others and that an average rate based on only a few loci, including one which accounts for nearly half of the observed mutations, is a statistic of very limited meaning." Although Ives does not mention it, these factors, the variation among loci, the small number of loci tested, and other limitations, for example the fact that the loci were not a random sample, were all pointed out in my 1951

publication. However, it is perhaps worth inquiring further into the meaning of the statistic. Of course the particular values of the means arrived at by Ives are based on the two unjustified procedures already discussed, namely: first, the comparison of unadjusted Drosophila spermatozoa mutation rates with mouse spermatogonia rates, and, second, the use of "F_1 results." His conclusion that the two species have "similar" radiation-induced mutation rates is, therefore, based primarily on these errors rather than on any limitation of the statistic. Comparing Alexander's spermatogonia results for tested mutations with the mouse results, there is no question of a highly significant difference between the mean mutation rates of the two species so far as the loci tested are concerned. Statistical tests of high sensitivity are not available for the more important question of whether the data indicate a significant difference in over-all mutation rates between the two species, assuming the two sets of loci to be, at least, random samples of comparable kinds of loci. Nevertheless, the application of a non-parametric test, namely, Pitman's (1937), does show a difference of this kind which is significant at the 3.9% level.[3] The following semi-quantitative considerations are also instructive. Five of the seven loci tested in the mouse spermatogonia gave induced mutation rates higher than the highest of the rates obtained by Alexander for the eight loci in Drosophila spermatogonia and also higher than the highest Drosophila spermatozoa rate, even without adjusting for the four-fold greater mean rate in spermatozoa. In the quotation cited earlier in this paragraph, and in another place in his paper, Ives emphasizes the high mutation rate at the *S* locus in the mouse, apparently implying that this locus in particular gives a spuriously high value to the mean mutation rate in the mouse. However, the rate at this locus is only a little over twice that at the locus with the next highest rate. This difference is not particularly noteworthy in view of the fact that the total range in the published data is from zero mutations at two loci to 25 mutations at the *S* locus. It remains to be seen whether there are many loci with rates as high as that at the *S* locus, but the fact that, in a sample of six other loci, three have rates between one-fifth and one-half as high indicates that the *S* locus may not be entirely out of line with the other loci. As was suggested in my 1951 publication, there could be unusual features about the *S* locus, but it would be wholly unjustified, in using the present data for the estimation of human hazards, to exclude the *S* locus simply because it has the highest mutation rate of the loci tested.

In further discussing the *S* locus, Ives makes an invalid comparison of it with the *scarlet* locus in Drosophila. Thus, in comparing his results with Alexander's, he makes the statement: "Most of the difference between those two rates is accountable by the difference in *scarlet*. In Russell's mouse study there was also one locus, *spotted*, which mutated frequently...." As has been pointed out, the "difference between those two rates" is attribut-

[3] I am grateful to Dr. A. W. Kimball of the Mathematics Panel of the Oak Ridge National Laboratory for suggesting this test and making the computations.

able mainly to Ives' erroneous way of calculating rates, namely, on the basis of "F_1 results." For the seven loci in Alexander's data that can be compared with Ives' results, *scarlet* "variants" formed the largest group in her "phenocopies and mimics" category, but these were variants that had been tested and found not be to mutations at the *scarlet* locus. Alexander found no *scarlet* locus mutations in her spermatogonia study, and, on the basis of tested mutations, *scarlet* was not the most mutable locus in her spermatozoa study. *Scarlet* was also not the most mutable locus in the results of Patterson, of Mickey (1954) and of Ives. Ives' comparison of *scarlet* in Drosophila with the *S* locus in the mouse is, therefore, invalid. The purpose for which Ives makes this comparison is not clear, but if he is implying that the *S*-locus mutations in the mouse could be mostly phenocopies or mimics, this is also invalid. All spotted variants classified as presumed *S*-locus mutations that have been tested, and these now include all the ones listed as untested in the 1951 publication, have proved to be mutations at the *S* locus.

Ives does not discuss in detail the original comparison of mouse and Drosophila mutation rates in my 1951 paper. However, in view of a statement he makes about the mouse data, and in view of what has been said in the present paper about "F_1 results," it seems desirable to reiterate the way in which the original comparison was made. It will also be of interest to consider this comparison in the light of information obtained subsequently. Ives' statement is: "Russell included in his fifty-four mutants a group of six which died before being tested for mimetic dominant mutation and a group of twelve which had not been tested at the time of publication, these eighteen cases being judged solely on the basis of the F_1 phenotype and Russell's very large previous experience with mouse mutations." This problem was discussed at length in my publication and the main reason, which Ives does not mention, for including these cases in the calculation of mutation rate was, as I then stated it: "The reliability of classification by phenotype has been confirmed by the allelism tests that have been made. In all of the first 26 presumed mutations adequately tested, the classification by phenotype has proved correct, and in the next 10 partially tested there is so far no evidence against the allelism assumed." Thus, there was strong evidence at that time that most, if not all, of the 48 viable variants classified by their phenotypes as presumed mutations at the specific loci would probably test to be such. (Since that time, all of them have indeed proved to be what they were presumed to be.)

The most extensive Drosophila data of a comparable kind at that time were those of Valencia and Muller (1949). These were published only in abstract form and it was unfortunately not possible to tell exactly whether or not the mutation rates given, which were based on mutations "from which stocks were obtained," could be compared directly with the tested mutations category in the mouse. Valencia and Muller did, however, give the further information that the mutations from which stocks were obtained represented "some 50 per cent of total." I, therefore, adopted the conservative

procedure, so far as demonstrating a difference between the species was concerned, and doubled the mutation rate given by Valencia and Muller. This, after making an allowance for the difference between spermatozoa and spermatogonia results, also, as it has turned out, conservative, was compared with the total rate in the mouse, that is, the rate obtained by including the variants not yet tested at that time and the six variants that died before testing. That this was indeed a conservative procedure is now shown by the fact that, while the ratio of total variants to mutations from which stocks could be obtained was 2 in the Valencia and Muller Drosophila data, it has turned out to be only 1.1 in the mouse. Thus, the original comparison gave a mouse to Drosophila mutation-rate ratio of 12.5; but if the mouse data could have been compared with the Drosophila data of Valencia and Muller on the basis of mutations from which stocks were obtained, and if we had taken the Drosophila spermatogonia mutation rate as being only one-quarter of the spermatozoa rate, the mouse rate would have come out 47 times higher than the Drosophila rate of Valencia and Muller.

This figure is considerably higher than the best estimate of the ratio of mouse to Drosophila mutation rates that will be presented later. One possible explanation, out of many, is that there may be a difference between mutation rates for autosomal and X-chromosome loci. Before leaving the discussion of the original comparison of mouse and Drosophila mutation rates, it may be pointed out that this possibility of a difference between mutation rates for autosomal and X-chromosome loci, a possibility greatly emphasized by Ives, was recognized in three ways. In the first place, some Drosophila data on autosomal loci ware considered in the comparison. Second, the comparison was made in a conservative way and the results were stated in cautious terms. Third, we initiated the investigation made by Alexander to obtain data that would be based on autosomal loci.

Since, in Alexander's data on Drosophila spermatogonia, we now have the most closely similar results for comparison with the mouse data, it is of interest at this time to examine the magnitude of the difference in rates. The mouse rate for tested mutations may be calculated on the basis of the viable variants reported in my 1951 publication since, as mentioned above, the ten that had been only partially tested at that time and the twelve presumed mutations that had not yet been tested have since been fully tested and all of them have proved to be mutations at the specific loci. This leaves the six mouse "mutants" that died before testing. If these are excluded for purposes of comparison with Alexander's data, the mouse radiation-induced mutation rate is reduced from the published figure of 25.0×10^{-5} to 22.1×10^{-5} per roentgen, per locus. Comparing this with Alexander's figure of 1.52×10^{-5} per roentgen, per locus for the Drosophila spermatogonia mutation rate gives an estimate of 15 as the ratio of the mouse rate to the Drosophila rate.

Since the Drosophila spermatogonia mutation rate for specific loci is based on only ten mutations, it is still of interest to compare the mouse rate with the Drosophila spermatozoa rate, particularly the new and inde-

pendent sets of information on specific loci in autosomes. Alexander's rate for 71 tested mutations in spermatozoa is 5.98×10^{-8} per roentgen, per locus. Mickey's (1954) rate for 32 mutations at the same eight loci is 3.35×10^{-8}. Patterson's rate (quoted by Alexander) for a total of 70 mutations at seven of the same loci and one other locus is 4.39×10^{-8}. In estimating spermatogonia rates from these spermatozoa rates, a step necessary before comparison with the mouse data, it would defeat out purpose of getting estimates independent of the limited information on specific loci rates in Drosophila spermatogonia if we were to use Alexander's estimate of the ratio of spermatozoa to spermatogonia rates. The information that comes closest to providing a satisfactory independent estimate of this ratio is that of Auerbach (1954). Table 1 in her publication gives the rate of mutation to recessive lethals on the second chromosome in broods of Drosophila coming from irradiated males mated for successive periods of three days each. Data from the last brood are presumed to be from irradiated spermatogonia. In Mickey's experiment the males were mated for six days following irradiation. His data may, therefore, be taken as being roughly equivalent to the first two broods of Auerbach's data. The ratio of the weighted mean mutation rate for these two broods compared with the rate in the last brood is 4.6. Dividing Mickey's rate by this, we come out with 0.73×10^{-8} per roentgen, per locus as an estimate of the spermatogonia rate in Drosophila. Dividing the mouse rate by this adjusted Drosophila rate gives a ratio of mouse to Drosophila rates of 30. In Alexander's experiment the males were mated for four days following irradiation. Taking this as corresponding to Auerbach's first brood and one-third of her second brood, we arrive at 3.7 as the figure with which to divide Alexander's spermatozoa rate. This gives an estimate of 1.6×10^{-8} per roentgen, per locus for the spermatogonia rate, and 14 as the ratio of the mouse to the Drosophila rate. These last figures are in excellent agreement with those obtained directly from Alexander's spermatogonia data. It may be noted that Alexander's data when compared with the mouse results give a more conservative estimate of the difference between mouse and Drosophila mutation rates than do Mickey's data. The mating period for the males is not given for Patterson's data.

Before long we hope to have adequate data on spermatozoa mutation rates in mice for direct comparison with the Drosophila spermatozoa rates. They were not obtained earlier because it was felt that it was far more important, from the human hazard point of view, to obtain spermatogonia rates. In this connection, Ives makes another error. He states: "In mice the mature sperm are so affected by the radiation that the zygotes resulting from them are abortive. Therefore one does not know the actual mutation rate at the visible loci of mice in mature sperm....." This is incorrect. At 600 r, dominant lethality in mice is not too great to permit determination of mutation rate in sperm. This has been known for over twenty years (see review by Russell, 1954).

Ives nowhere discusses the application of the mouse-Drosophila comparison to the estimation of human hazards. In my 1951 paper the species

comparison was made primarily with this estimation in mind and was, in fact, discussed under the section heading "Human Hazards." I concluded this section with the following statement: "From a cautious scientific point of view, it would be unwise to generalize.... but, from the point of view of those concerned with the immediate problems of protection in man, it would be risky to ignore the indication that estimates of human hazards based on Drosophila mutation rates may be too low." I still hold this point of view. Comparisons between species as different as Drosophila and mice are difficult. There are many possible complicating factors additional to those already discussed. To mention only one, the relation between mutation rate and dose, for recovered mutants from spermatogonia in the mouse, is proving to be non-linear. Because of the possible complicating factors, it would still be unwise to generalize about the relative rates of mutation in Drosophila and mice, although one can perhaps be less conservative than I was in my 1951 publication. However, because of the human hazards, there is a pressing need to draw the best conclusion one possibly can. It is, therefore, important that, regardless of the difficulties, the comparison between Drosophila and mice be made as meaningfully and as accurately as possible at the present time. It is on this basis that Ives' arguments and conclusions have been questioned, and not because of any belief that a final answer has been reached as to the relative mutation rates of the two species.

A casual reader of Ives' paper, if he accepts Ives' conclusion that "the radiation-induced mutation rate per r per locus appears to be similar in flies and mice," might think that this invalidates the conclusion of my 1951 paper that "estimates of human hazards based on Drosophila mutation rates may be too low." Such is not the case. Ives does not have any serious objections to the figure of 25×10^{-6} per roentgen, per locus, for the mouse mutation rate; he is simply "calculating" the Drosophila rate in a way that raises it closer to the mouse rate. But the Drosophila figure that was being used most commonly to estimate human hazards at the time when my 1951 paper suggested more caution, was not the high figure of 14×10^{-6} or 23×10^{-6} per roentgen, per locus, arrived at by Ives, but 3×10^{-6}. Furthermore, since this was based on Drosophila spermatozoa, it was also assumed that there might be a safety factor of two or so in applying this to human spermatogonia. Thus, regardless of whether Ives' conclusions are correct or not, and the burden of this paper has been to demonstrate that they are not, he has not shown any reason to relax from the extra caution that the mouse results originally indicated should be taken.

CONCLUSIONS

In an earlier paper (Russell, 1951) it was concluded that comparison of our data on radiation-induced mutation rates in mice with similar data from Drosophila, particularly those of Valencia and Muller (1949), indicated a considerably higher mean induced mutation rate in the mouse. New information on Drosophila supports this conclusion. The Drosophila mutation rates now most suitable for comparison with the mouse are those obtained pri-

marily for this purpose by Alexander (1954). These rates, like the mouse rates, are for mutations at specific loci on autosomes and they were obtained by essentially the same genetic technique and method of scoring the mutations as had been used on the mice. Furthermore, the X-ray machine and method of dosimetry were the same as those used in the mouse experiments. Perhaps the most important new feature of this Drosophila information is that it was obtained from irradiation of the same cell stage as that investigated in the mouse, namely, the spermatogonial stage. Comparing the mouse radiation-induced mutation rate based on genetically tested mutations with these comparable data in Drosophila gives an estimate of 15 as the ratio of the mouse rate to the Drosophila rate.

The general magnitude of this ratio is also supported by several recent estimates of Drosophila mutation rates for autosomal loci in postspermatogonial cell stages, provided due regard is paid to the differences between cell stages. Ives' (1954) conclusion that the radiation-induced mutation rate "appears to be similar in flies and mice" is disputed on several grounds.

Comparisons between species as different as Drosophila and the mouse are difficult. It is not maintained that a final answer has been reached on the relative radiation-induced mutation rates of the two species. However, the new Drosophila data would seem to reinforce the conclusion reached in my 1951 paper that "from the point of view of those concerned with the immediate problems of protection in man, it would be risky to ignore the indication that estimates of human hazards based on Drosophila mutation rates may be too low."

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8